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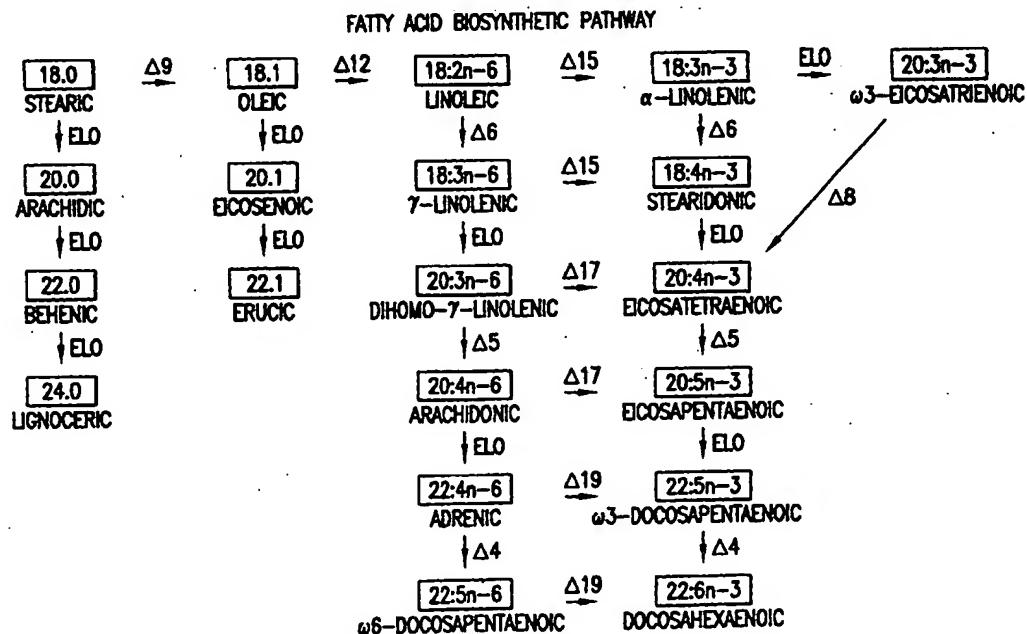
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(54) Title: Δ4-DESATURASE GENES AND USES THEREOF



(57) Abstract: The subject invention relates to the identification of genes involved in the desaturation of polyunsaturated fatty acids at carbon 4 (i.e., "Δ4-desaturase"). In particular, Δ4-desaturase may be utilized, for example, in the conversion of adrenic acid to ω6-docosapentaenoic acid and in the conversion of ω3-docosapentaenoic acid to docosahexaenoic acid. The polyunsaturated fatty acids produced by use of the enzyme may be added to pharmaceutical compositions, nutritional compositions, animal feeds, as well as other products such as cosmetics.

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**Δ4-DESATURASE GENES AND USES THEREOF**BACKGROUND OF THE INVENTION

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Technical Field

The subject invention relates to the identification and isolation of genes that encode enzymes (e.g., *Thraustochytrium aureum* Δ4-desaturase, *Schizochytrium aggregatum* Δ4-desaturase and *Isochrysis galbana* Δ4-desaturase) involved in the synthesis of polyunsaturated fatty acids and to uses thereof. In particular, Δ4-desaturase catalyzes the conversion of, for example, adrenic acid (22:4n-6) to ω6-docosapentaenoic acid (22:5n-6) and the conversion of ω3-docosapentaenoic acid (22:5n-3) to docosahexaenoic acid (22:6n-3). The converted products may then be utilized as substrates in the production of other polyunsaturated fatty acids (PUFAs). The product or other polyunsaturated fatty acids may be added to pharmaceutical compositions, nutritional composition, animal feeds as well as other products such as cosmetics.

Background Information

Desaturases are critical in the production of long-chain polyunsaturated fatty acids that have many important functions. For example, polyunsaturated fatty acids (PUFAs) are important components of the plasma membrane of a cell, where they are found in the form of phospholipids. They also serve as precursors to mammalian prostacyclins, eicosanoids, leukotrienes and prostaglandins.

Additionally, PUFAs are necessary for the proper development of the developing infant brain as well as for tissue formation and repair. In view of the biological significance of PUFAs, attempts are being made to produce them, as well as intermediates leading to their production, in an efficient manner.

A number of enzymes, most notably desaturase and elongases, are involved in PUFA biosynthesis (see Figure 1). For example, elongase (elo) catalyzes the conversion of  $\gamma$ -linolenic acid (GLA) to dihomo- $\gamma$ -linolenic acid (DGLA) and of 5 stearidonic acid (18:4n-3) to (n-3)-eicosatetraenoic acid (20:4n-3). Linoleic acid (LA, 18:2n-9,12 or 18:2n-6) is produced from oleic acid (18:1- $\Delta$ 9) by a  $\Delta$ 12-desaturase. GLA (18:3n-6,9,12) is produced from linoleic acid by a  $\Delta$ 6-desaturase.

10 It must be noted that animals cannot desaturate beyond the  $\Delta$ 9 position and therefore cannot convert oleic acid into linoleic acid. Likewise,  $\gamma$ -linolenic acid (ALA, 18:3n-9,12,15) cannot be synthesized by mammals. However,  $\gamma$ -linolenic acid can be converted to stearidonic acid (STA, 15 18:4n-6,9,12,15) by a  $\Delta$ 6-desaturase (see PCT publication WO 96/13591 and The FASEB Journal, Abstracts, Part I, Abstract 3093, page A532 (Experimental Biology 98, San Francisco, CA, April 18-22, 1998); see also U.S. Patent No. 5,552,306), followed by elongation to (n-3)-eicosatetraenoic acid (20:4n-20 8,11,14,17) in mammals and algae. This polyunsaturated fatty acid (i.e., 20:4n-8,11,14,17) can then be converted to eicosapentaenoic acid (EPA, 20:5n-5,8,11,14,17) by a  $\Delta$ 5-desaturase. EPA can then, in turn, be converted to  $\omega$ 3-docosapentaenoic acid (22:5n-3) by an elongase. Isolation of 25 an enzyme or its encoding gene, responsible for conversion of  $\omega$ 3-docosapentaenoic acid to docosahexaenoic acid (22:6n-3) has never been reported. Two pathways for this conversion have been proposed (see Figure 1 and Sprecher, H., Curr. Opin. Clin. Nutr. Metab. Care, Vol.2, p. 135-138, 1999). One of 30 them involves a single enzyme, a  $\Delta$ 4-desaturase such as that of the present invention. In the n-6 pathway, dietary linoleic acid may be converted to adrenic acid through a series of desaturation and elongation steps in mammals, (see Figure 1). Production of  $\omega$ 6-docosapentaenoic acid from adrenic acid is

postulated to be mediated by the  $\Delta 6$ -desaturase discussed above.

Other eukaryotes, including fungi and plants, have enzymes which desaturate at carbon 12 (see PCT publication WO 94/11516 and U.S. Patent No. 5,443,974) and carbon 15 (see PCT publication WO 93/11245). The major polyunsaturated fatty acids of animals therefore are either derived from diet and/or from desaturation and elongation of linoleic acid or  $\gamma$ -linolenic acid. In view of these difficulties, it is of significant interest to isolate genes involved in PUFA synthesis from species that naturally produce these fatty acids and to express these genes in a microbial, plant, or animal system which can be altered to provide production of commercial quantities of one or more PUFAs.

In view of the above discussion, there is a definite need for the  $\Delta 4$ -desaturase enzyme, the respective genes encoding this enzyme, as well as recombinant methods of producing this enzyme. Additionally, a need exists for oils containing levels of PUFAs beyond those naturally present as well as those enriched in novel PUFAs. Such oils can only be made by isolation and expression of the  $\Delta 4$ -desaturase gene(s).

All U.S. patents and publications referred to herein are hereby incorporated in their entirety by reference.

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#### SUMMARY OF THE INVENTION

The present invention includes an isolated nucleotide sequence or fragment thereof comprising or complementary to a nucleotide sequence encoding a polypeptide having desaturase activity. The amino acid sequence of the polypeptide has at least 50% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:37, SEQ ID NO:46 and SEQ ID NO:55. Also, in particular, the present invention encompasses an isolated nucleotide sequence or fragment thereof comprising or complementary to a nucleotide sequence encoding a

polypeptide having desaturase activity, wherein the amino acid sequence of said polypeptide has at least 30% identity to the amino acid sequence of SEQ ID NO:55.

Additionally, the present invention encompasses an isolated nucleotide sequence or fragment thereof comprising or complementary to a nucleotide sequence having at least 50% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:36, SEQ ID NO:45 and SEQ ID NO:54. In particular, the present invention includes an isolated nucleotide sequence or fragment thereof comprising or complementary to a nucleotide sequence having at least 40% identity to the nucleotide sequence of SEQ ID NO:54.

Each of the sequences described above encodes a functionally active desaturase that utilizes a monounsaturated or polyunsaturated fatty acid as a substrate. The nucleotide sequences may be derived for example, from a fungus or an algae. In particular, when the nucleotide sequence comprises SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17 or SEQ ID NO:45, may be derived, for example, from the fungus *Thraustochytrium aureum*. The sequence comprising SEQ ID NO:36 may be derived, for example, from the fungus *Schizochytrium aggregatum*. The sequence comprising SEQ ID NO:54 may be derived, for example, from the algae *Isochrysis galbana*. The present invention also includes purified protein and fragments thereof encoded by the above-referenced nucleotide sequences.

In particular, the present invention also includes a purified polypeptide which desaturates polyunsaturated fatty acids at carbon 4 and has an amino acid sequence having at least 50% identity to an amino acid sequence selected from the group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:37, SEQ ID NO:46 and SEQ ID NO:55. In particular, the present invention also includes a purified polypeptide which desaturates polyunsaturated fatty acids at

carbon 4 and has an amino acid sequence having at least 30% identity to the amino acid sequence of SEQ ID NO:55.

Additionally, the present invention includes a Method of producing a desaturase comprising the steps of:

5 isolating a nucleotide sequence comprising or complementary to a nucleotide sequence encoding a polypeptide having an amino acid sequence having at least 50% identity to an amino acid sequence selected from the group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:37, SEQ ID  
10 NO:46 and SEQ ID NO:55 (or at least 30% identity to the amino acid sequence of SEQ ID NO:55) or having at least 50% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:36, SEQ ID NO:45 and SEQ ID NO:54 (or having,  
15 in particular, at least 40% sequence identity to SEQ ID NO:54); constructing a vector comprising: i) the isolated nucleotide sequence operably linked to ii) a promoter; and introducing said vector into a host cell for a time and under conditions sufficient for expression of the desaturase. The  
20 host cell may be, for example, a eukaryotic cell or a prokaryotic cell. In particular, the prokaryotic cell may be, for example, E. coli, cyanobacteria or B. subtilis. The eukaryotic cell may be, for example, a mammalian cell, an insect cell, a plant cell or a fungal cell (e.g., a yeast cell  
25 such as Saccharomyces cerevisiae, Saccharomyces carlsbergensis, Candida spp., Lipomyces starkey, Yarrowia lipolytica, Kluyveromyces spp., Hansenula spp., Trichoderma spp. or Pichia spp.).

Moreover, the present invention also includes a vector comprising: an isolated nucleotide sequence comprising or complementary to a nucleotide sequence encoding a polypeptide having an amino acid sequence having at least 50% amino acid identity to an amino acid sequence selected from the group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:37, SEQ ID NO:46 and SEQ ID NO:55 (or, in particular, at least 30% amino acid identity to SEQ ID NO:55)

or having at least 50% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:36, SEQ ID NO:45 and SEQ ID NO:54 (or, in particular, at least 40% 5 identity to SEQ ID NO:54), operably linked to a promoter. The invention also includes a host cell comprising this vector. The host cell may be, for example, a eukaryotic cell or a prokaryotic cell. Suitable eukaryotic cells and prokaryotic cells are as defined above.

10 Moreover, the present invention also includes a plant cell, plant or plant tissue comprising the above vector, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid by the plant cell, plant or plant tissue. The polyunsaturated fatty 15 acid may be, for example, selected from the group consisting of  $\omega$ 6-docosapentaenoic acid or docosahexaenoic acid. The invention also includes one or more plant oils or acids expressed by the above plant cell, plant or plant tissue.

Additionally, the present invention also encompasses a 20 transgenic plant comprising the above vector, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid in seeds of the transgenic plant.

The present invention also includes a method ("first 25 method") for producing a polyunsaturated fatty acid comprising the steps of: isolating a nucleotide sequence comprising or complementary to a nucleotide sequence encoding a polypeptide having an amino acid sequence having at least 50% amino acid sequence identity to an amino acid sequence selected from the 30 group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:37, SEQ ID NO:46 and SEQ ID NO:55 (and, in particular, at least 30% amino acid sequence identity to SEQ ID NO:55) or having at least 50% sequence identity to a nucleotide sequence selected from the group consisting of SEQ 35 ID NO:14, SEQ ID NO:15, SEQ ID NO:16 and SEQ ID NO:17, SEQ ID

NO:36, SEQ ID NO:45, and SEQ ID NO:54 (and, in particular, at least 40% with respect to SEQ ID NO:54); constructing a vector comprising the isolated nucleotide sequence; introducing the vector into a host cell for a time and under conditions

5 sufficient for expression of  $\Delta 4$ -desaturase; and exposing the expressed  $\Delta 4$ -desaturase to a substrate polyunsaturated fatty acid in order to convert the substrate to a product polyunsaturated fatty acid. The substrate polyunsaturated fatty acid may be, for example, adrenic acid or  $\omega 3$ -

10 docospentaenoic acid, and the product polyunsaturated fatty acid may be, for example,  $\omega 6$ -docosapentaenoic acid or docosahexaenoic acid, respectively. This method may further comprise the step of exposing the product polyunsaturated fatty acid to another enzyme (e.g., a  $\Delta 4$ -desaturase, an

15 elongase or another desaturase) in order to convert the product polyunsaturated fatty acid to another polyunsaturated fatty acid (i.e., "second" method). In this method containing the additional step (i.e., "second" method), the product polyunsaturated fatty acid may be, for example,  $\omega 6$ -

20 docosapentaenoic acid, and the "another" polyunsaturated fatty acid may be docosahexaenoic acid.

Also, the present invention includes a method of producing a polyunsaturated fatty acid comprising the steps of: exposing a substrate polyunsaturated fatty acid to one or 25 more enzymes selected from the group consisting of a desaturase and an elongase in order to convert the substrate to a product polyunsaturated fatty acid; and exposing the product polyunsaturated fatty acid to a  $\Delta 4$ -desaturase comprising an amino acid sequence selected

30 from the group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:37, SEQ ID NO:46 and SEQ ID NO:55, in order to convert the product polyunsaturated fatty acid to a final product polyunsaturated fatty acid.

For example, a substrate polyunsaturated fatty acid

35 (e.g., eicosapentaenoic acid) may be exposed to an elongase or

desaturase (e.g., MEL04 or other elongases or desaturases of significance in the biosynthetic pathway) in order to convert the substrate to a product polyunsaturated fatty acid (e.g.,  $\omega$ 3-docosapentaenoic acid). The product polyunsaturated fatty acid may then be converted to a "final" product polyunsaturated fatty acid (e.g., docosahexaenoic acid) by exposure to the  $\Delta$ 4-desaturase of the present invention (see Figure 1). Thus, the  $\Delta$ 4-desaturase is utilized in the last step of the method in order to create the "final" desired product. As another example, one may expose linoleic acid to a  $\Delta$ 6-desaturase in order to create  $\gamma$ -linolenic acid (GLA), and then expose the GLA to an elongase and then to a  $\Delta$ 5-desaturase in order to create arachidonic acid (AA). The AA may then be exposed to an elongase in order to convert it to adrenic acid. Finally, the adrenic acid may be exposed to  $\Delta$ 4-desaturase in order to convert it to  $\omega$ 6-docosapentaenoic acid (see Figure 1). Thus, the method involves the utilization of a linoleic acid substrate and a series of desaturase and elongase enzymes, in addition to the  $\Delta$ 4-desaturase, in order to arrive at the final product. By use of a similar method, one may also convert the substrate PUFA,  $\gamma$ -linolenic acid to docosahexaenoic acid. Again, various desaturases and elongase are used to ultimately arrive at  $\omega$ 3-docosapentaenoic acid which is then exposed to one or more of the  $\Delta$ 4-desaturases of the present invention in order to convert it to docosahexaenoic acid. (Possible substrates include those shown in Figure 1, for example, linoleic acid,  $\gamma$ -linolenic acid, stearidonic acid, arachidonic acid, dihomo- $\gamma$ -linolenic acid, adrenic acid, eicosapentaenoic acid and eicosatetraenoic acid.)

The present invention also encompasses a composition comprising at least one polyunsaturated fatty acid selected from the group consisting of the "product" polyunsaturated fatty acid produced according to the methods described above and the "another" polyunsaturated fatty acid produced

according to the methods described above. The product polyunsaturated fatty acid may be, for example,  $\omega 6$ -docosapentaenoic acid or docosahexaenoic acid. The another polyunsaturated fatty acid may be, for example, 5 docosahexaenoic acid.

Additionally, the present invention encompasses a method of preventing or treating a condition caused by insufficient intake of polyunsaturated fatty acids comprising administering to the patient the composition above in an amount sufficient 10 to effect prevention or treatment.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the fatty acid biosynthetic pathway and the role of  $\Delta 4$ -desaturase in this pathway.

15 Figure 2 illustrates an amino acid comparison of  $\Delta 4$ -desaturases produced by four different plasmids (SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20 and SEQ ID NO:21).

Figure 3 illustrates the nucleotide sequence encoding  $\Delta 4$ -desaturase of *Thraustochytrium aureum* (ATCC 34304) from 20 plasmid pRTA5 (SEQ ID NO:14).

Figure 4 illustrates the nucleotide sequence encoding  $\Delta 4$ -desaturase of *Thraustochytrium aureum* (ATCC 34304) from plasmid pRTA6 (SEQ ID NO:15).

25 Figure 5 illustrates the nucleotide sequence encoding  $\Delta 4$ -desaturase of *Thraustochytrium aureum* (ATCC 34304) from plasmid pRTA7 (SEQ ID NO:16).

Figure 6 illustrates the nucleotide sequence encoding 30 encoding  $\Delta 4$ -desaturase of *Thraustochytrium aureum* (ATCC 34304) from plasmid pRTA8 (SEQ ID NO:17).

Figure 7 illustrates the amino acid sequence of  $\Delta 4$ -desaturase of *Thraustochytrium aureum* (ATCC 34304) from 35 plasmid pRTA5 (SEQ ID NO:18).

Figure 8 illustrates the amino acid sequence of  $\Delta 4$ -desaturase of *Thraustochytrium aureum* (ATCC 34304) from plasmid pRTA6 (SEQ ID NO:19).

Figure 9 illustrates the amino acid sequence of  $\Delta 4$ -desaturase of *Thraustochytrium aureum* (ATCC 34304) from plasmid pRTA7 (SEQ ID NO:20).

Figure 10 illustrates the amino acid sequence of  $\Delta 4$ -desaturase of *Thraustochytrium aureum* (ATCC 34304) from plasmid pRTA8 (SEQ ID NO:21).

Figure 11 illustrates the nucleotide and amino acid sequences described herein.

Figure 12 illustrates the amino acid sequence encoded by the elongase gene MEL04 from a mouse.

Figure 13 illustrates the DNA sequence of the putative  $\Delta 4$ -desaturase ssa.con (SEQ ID NO:24) generated from clones saa9 and saa5 from *S. aggregatum* (ATCC 28209) (see Example VI).

Figure 14 illustrates the amino acid sequence (SEQ ID NO:25) of the putative  $\Delta 4$ -desaturase encoded by the ssa.con DNA sequence from *S. aggregatum* (ATCC 28209) (see Example VI).

Figure 15 illustrates the alignment of the amino acids derived from the translation of the open reading frames of ssa.con DNA from *S. aggregatum* (ATCC 28209) (SEQ ID NO:25) and pRTA7 (SEQ ID NO:68) (see Example VI).

Figure 16 illustrates the DNA sequence of the  $\Delta 4$ -desaturase from pRSA1 (SEQ ID NO:36) *S. aggregatum* (ATCC 28209) (see Example VII).

Figure 17 illustrates the amino acid sequence (SEQ ID NO:37) of the  $\Delta 4$ -desaturase encoded by the pRSA1 DNA sequence from *S. aggregatum* (ATCC 28209) (see Example VII).

Figure 18 illustrates the DNA sequence of the  $\Delta 4$ -desaturase from pRTA11 (SEQ ID NO:45) *T. aureum* (BICC 7091) (see Example VII).

Figure 19 illustrates the amino acid sequence (SEQ ID NO:46) of the putative  $\Delta$ 4-desaturase encoded by the pRTA11 DNA sequence from *T. aureum* (BICC 7091) (see Example VII).

5 Figure 20 illustrates the DNA sequence of the  $\Delta$ 4-desaturase from *Isochrysis galbana* (CCMP1323) (SEQ ID NO: 54) present in clone pRIG6 (see Example IX).

Figure 21 illustrates the amino acid sequence (SEQ ID NO:55) of the  $\Delta$ 4-desaturase encoded by the pRIG6 DNA sequence from *Isochrysis galbana* (CCMP1323) (see Example IX).

10 Figure 22 illustrates the percent identity between the novel  $\Delta$ 4-desaturase from *I. galbana* (CCMP 1323) (SEQ ID NO:69) and the  $\Delta$ 4-desaturase from *Thraustochytrium aureum* (ATCC 34304) (SEQ ID NO:70).

15 DETAILED DESCRIPTION OF THE INVENTION

The subject invention relates to the nucleotide and translated amino acid sequences of the  $\Delta$ 4-desaturase gene derived from the fungus *Thraustochytrium aureum* (BICC 7091), the fungus *Schizochytrium aggregatum*, and the algae *Isochrysis galbana*. Furthermore, the subject invention also includes uses of these genes and of the enzymes encoded by these genes. For example, the genes and corresponding enzymes may be used in the production of polyunsaturated fatty acids such as, for instance,  $\omega$ 6-docosapentaenoic acid and/or docosahexaenoic acid which may be added to pharmaceutical compositions, nutritional compositions and to other valuable products.

The  $\Delta$ 4-Desaturase Genes and Enzymes Encoded Thereby

As noted above, the enzymes encoded by the  $\Delta$ 4-desaturase genes of the present invention are essential in the production of highly unsaturated polyunsaturated fatty acids having a length greater than 22 carbons. The nucleotide sequences of the isolated *Thraustochytrium aureum*  $\Delta$ 4-desaturase genes, which differed based upon the plasmid created (see Example

III), are shown in Figures 3-6, and the amino acid sequences of the corresponding purified proteins are shown in Figure 7-10. An additional, isolated *T. aureum* nucleotide sequence is shown in Figure 18 (see Example VII), and the encoded amino acid sequence is shown in Figure 19. The nucleotide sequences of the isolated *Schizochytrium aggregatum* Δ4-desaturase sequences are shown in Figures 13 and 16, and the encoded amino acid sequences are shown in Figures 14 and 17, respectively. Additionally, the nucleotide sequences of the isolated *Isochrysis galbana* Δ4-desaturase sequence is shown in Figure 20, and the amino acid sequence encoded by the nucleotide sequence is shown in Figure 21.

As an example of the importance of the genes of the present invention, the isolated Δ4-desaturase genes convert adrenic acid to ω6-docosapentaenoic acid or convert ω3-docosapentaenoic acid to docosahexaenoic acid.

It should be noted that the present invention also encompasses isolated nucleotide sequences (and the corresponding encoded proteins) having sequences comprising, corresponding to, identical to, or complementary to at least about 50%, preferably at least about 60%, and more preferably at least about 70%, even more preferably at least about 80%, and most preferably at least about 90% sequence identity to SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16 or SEQ ID NO:17 (i.e., the nucleotide sequences of the Δ4-desaturase gene of *Thraustochytrium aureum* (ATCC 34304)), to SEQ ID NO:36 (i.e., the nucleotide sequence of the Δ4-desaturase gene of *Schizochytrium aggregatum* (ATCC 28209) or to SEQ ID NO:45 (i.e., the nucleotide sequence of the Δ4-desaturase gene of *Thraustochytrium aureum* (BICC 7091)) or to SEQ ID NO:54 (i.e., the nucleotide sequence of the Δ4-desaturase gene of *Isochrysis galabana*), all described herein. With respect to the *I. galbana* sequence, in particular, the present invention also encompasses nucleotide sequences (and the corresponding encoded proteins) having sequences comprising, corresponding

to, identical to, or complementary to at least 40%, more preferably at least 60%, even more preferably at least 80%, and most preferably at least 90% of the nucleotide sequence of SEQ ID NO:54. (All integers between 40% and 100% are also 5 considered to be within the scope of the present invention with respect to percent identity.) Such sequences may be derived from any source, either isolated from a natural source, or produced via a semi-synthetic route, or synthesized *de novo*. In particular, such sequences may be isolated or 10 derived from sources other than described in the examples (e.g., bacteria, fungus, algae, C. elegans, mouse or human).

Furthermore, the present invention also encompasses fragments and derivatives of the nucleotide sequences of the present invention (i.e., SEQ ID NO:14, SEQ ID NO:15, SEQ ID 15 NO:16, SEQ ID NO:17, SEQ ID NO:36, SEQ ID NO:45 or SEQ ID NO:54), as well as of the sequences derived from other sources, and having the above-described complementarity, identity or correspondence. Functional equivalents of the above full length sequences and fragments (i.e., sequences 20 having  $\Delta 4$ -desaturase activity, as appropriate) are also encompassed by the present invention.

For purposes of the present invention, a "fragment" is of a nucleotide sequence is defined as a contiguous sequence of approximately at least 6, preferably at least about 8, more 25 preferably at least about 10 nucleotides, and even more preferably at least about 15 nucleotides corresponding to a region of the specified nucleotide sequence.

The invention also includes a purified polypeptide which desaturates polyunsaturated fatty acids at the carbon 4 30 position and has at least about 50% amino acid similarity or identity, preferably at least about 60% amino acid similarity or identity, more preferably at least about 70% amino acid similarity or identity, even more preferably at least about 80% amino acid similarity or identity and most preferably at 35 least 90% amino acid similarity or identity to the amino acid

sequences (i.e., SEQ ID NO:18 (shown in Figure 7), SEQ ID NO:19 (shown in Figure 8), SEQ ID NO:20 (shown in Figure 9), SEQ ID NO:21 (shown in Figure 10), SEQ ID NO:37 (shown in Figure 17), SEQ ID NO:46 (shown in Figure 19) and SEQ ID NO:55 (shown in Figure 21) of the above-noted proteins which are, in turn, encoded by the above-described nucleotide sequences. In particular, with respect to the amino acid sequence of the *I. galbana* Δ4-desaturase, the present invention encompasses includes a purified polypeptide which desaturates polyunsaturated fatty acids at the carbon 4 position and has at least about 30% amino acid similarity or identity, preferably at least about 50% amino acid similarity or identity, more preferably at least about 70% amino acid similarity or identity, and most preferably at least about 90% amino acid similarity or identity to the amino acid sequence of SEQ ID NO:55 (i.e., the amino acid sequence of the *I. galbana* Δ4-desaturase shown in Figure 21). (All integers between 30% and 100% similarity or identity are also included within the scope of the present invention.)

The term "identity" refers to the relatedness of two sequences on a nucleotide-by-nucleotide basis over a particular comparison window or segment. Thus, identity is defined as the degree of sameness, correspondence or equivalence between the same strands (either sense or antisense) of two DNA segments (or two amino acid sequences). "Percentage of sequence identity" is calculated by comparing two optimally aligned sequences over a particular region, determining the number of positions at which the identical base or amino acid occurs in both sequences in order to yield the number of matched positions, dividing the number of such positions by the total number of positions in the segment being compared and multiplying the result by 100. Optimal alignment of sequences may be conducted by the algorithm of Smith & Waterman, Appl. Math. 2:482 (1981), by the algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the

method of Pearson & Lipman, Proc. Natl. Acad. Sci. (USA) 85:2444 (1988) and by computer programs which implement the relevant algorithms (e.g., Clustal Macaw Pileup (<http://cmgm.stanford.edu/biochem218/11Multiple.pdf>; Higgins et al., CABIOS. 5:151-153 (1989)), FASTDB (Intelligenetics), BLAST (National Center for Biomedical Information; Altschul et al., Nucleic Acids Research 25:3389-3402 (1997)), PILEUP (Genetics Computer Group, Madison, WI) or GAP, BESTFIT, FASTA and TFASTA (Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, Madison, WI). (See U.S. Patent No. 5,912,120.)

For purposes of the present invention, "complementarity" is defined as the degree of relatedness between two DNA segments. It is determined by measuring the ability of the sense strand of one DNA segment to hybridize with the antisense strand of the other DNA segment, under appropriate conditions, to form a double helix. A "complement" is defined as a sequence which pairs to a given sequence based upon the canonic base-pairing rules. For example, a sequence A-G-T in one nucleotide strand is "complementary" to T-C-A in the other strand.

In the double helix, adenine appears in one strand, thymine appears in the other strand. Similarly, wherever guanine is found in one strand, cytosine is found in the other. The greater the relatedness between the nucleotide sequences of two DNA segments, the greater the ability to form hybrid duplexes between the strands of the two DNA segments.

"Similarity" between two amino acid sequences is defined as the presence of a series of identical as well as conserved amino acid residues in both sequences. The higher the degree of similarity between two amino acid sequences, the higher the correspondence, sameness or equivalence of the two sequences. ("Identity between two amino acid sequences is defined as the presence of a series of exactly alike or invariant amino acid residues in both sequences.) The definitions of

"complementarity", "identity" and "similarity" are well known to those of ordinary skill in the art.

"Encoded by" refers to a nucleic acid sequence which codes for a polypeptide sequence, wherein the polypeptide sequence or a portion thereof contains an amino acid sequence of at least 3 amino acids, more preferably at least 8 amino acids, and even more preferably at least 15 amino acids from a polypeptide encoded by the nucleic acid sequence.

The present invention also encompasses an isolated nucleotide sequence which encodes PUFA desaturase activity and that is hybridizable, under moderately stringent conditions, to a nucleic acid having a nucleotide sequence comprising or complementary to the nucleotide sequences described above. A nucleic acid molecule is "hybridizable" to another nucleic acid molecule when a single-stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and ionic strength (see Sambrook et al., "Molecular Cloning: A Laboratory Manual, Second Edition (1989), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York)). The conditions of temperature and ionic strength determine the "stringency" of the hybridization. "Hybridization" requires that two nucleic acids contain complementary sequences. However, depending on the stringency of the hybridization, mismatches between bases may occur. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation. Such variables are well known in the art. More specifically, the greater the degree of similarity or homology between two nucleotide sequences, the greater the value of  $T_m$  for hybrids of nucleic acids having those sequences. For hybrids of greater than 100 nucleotides in length, equations for calculating  $T_m$  have been derived (see Sambrook et al., *supra*). For hybridization with shorter nucleic acids, the position of mismatches becomes more

important, and the length of the oligonucleotide determines its specificity (see Sambrook et al., *supra*).

As used herein, an "isolated nucleic acid fragment or sequence" is a polymer of RNA or DNA that is single- or 5 double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases. An isolated nucleic acid fragment in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA or synthetic DNA. (A "fragment" of a specified polynucleotide refers to a 10 polynucleotide sequence which comprises a contiguous sequence of approximately at least about 6 nucleotides, preferably at least about 8 nucleotides, more preferably at least about 10 nucleotides, and even more preferably at least about 15 nucleotides, and most preferable at least about 25 nucleotides 15 identical or complementary to a region of the specified nucleotide sequence.) Nucleotides (usually found in their 5'-monophosphate form) are referred to by their single letter designation as follows: "A" for adenylate or deoxyadenylate (for RNA or DNA, respectively), "C" for cytidylate or 20 deoxycytidylate, "G" for guanylate or deoxyguanylate, "U" for uridylate, "T" for deoxythymidylate, "R" for purines (A or G), "Y" for pyrimidines (C or T), "K" for G or T, "H" for A or C or T, "I" for inosine, and "N" for any nucleotide.

The terms "fragment or subfragment that is functionally 25 equivalent" and "functionally equivalent fragment or subfragment" are used interchangeably herein. These terms refer to a portion or subsequence of an isolated nucleic acid fragment in which the ability to alter gene expression or produce a certain phenotype is retained whether or not the 30 fragment or subfragment encodes an active enzyme. For example, the fragment or subfragment can be used in the design of chimeric constructs to produce the desired phenotype in a transformed plant. Chimeric constructs can be designed for use in co-suppression or antisense by linking a nucleic acid 35 fragment or subfragment thereof, whether or not it encodes an

active enzyme, in the appropriate orientation relative to a plant promoter sequence.

The terms "homology", "homologous", "substantially similar" and "corresponding substantially" are used 5 interchangeably herein. They refer to nucleic acid fragments wherein changes in one or more nucleotide bases does not affect the ability of the nucleic acid fragment to mediate gene expression or produce a certain phenotype. These terms also refer to modifications of the nucleic acid fragments of 10 the instant invention such as deletion or insertion of one or more nucleotides that do not substantially alter the functional properties of the resulting nucleic acid fragment relative to the initial, unmodified fragment. It is therefore understood, as those skilled in the art will appreciate, that 15 the invention encompasses more than the specific exemplary sequences.

"Gene" refers to a nucleic acid fragment that expresses a specific protein, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding 20 sequences) the coding sequence.

"Native gene" refers to a gene as found in nature with its own regulatory sequences. In contrast, "chimeric construct" refers to a combination of nucleic acid fragments that are not normally found together in nature. Accordingly, a chimeric 25 construct may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that normally found in nature. (The term "isolated" means that the 30 sequence is removed from its natural environment.)

A "foreign" gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric

constructs. A "transgene" is a gene that has been introduced into the genome by a transformation procedure.

"Coding sequence" refers to a DNA sequence that codes for a specific amino acid sequence. "Regulatory sequences" refer 5 to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include, but are 10 not limited to, promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

"Promoter" refers to a DNA sequence capable of controlling the expression of a coding sequence or functional RNA. The promoter sequence consists of proximal and more 15 distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an "enhancer" is a DNA sequence which can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter.

20 Promoter sequences can also be located within the transcribed portions of genes, and/or downstream of the transcribed sequences. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise 25 synthetic DNA segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. Promoters which cause a gene to be 30 expressed in most host cell types at most times are commonly referred to as "constitutive promoters". New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro and Goldberg, (1989) *Biochemistry of Plants* 35 15:1-82. It is further recognized that since in most cases

the exact boundaries of regulatory sequences have not been completely defined, DNA fragments of some variation may have identical promoter activity.

An "intron" is an intervening sequence in a gene that 5 does not encode a portion of the protein sequence. Thus, such sequences are transcribed into RNA but are then excised and are not translated. The term is also used for the excised RNA sequences. An "exon" is a portion of the gene sequence that 10 is transcribed and is found in the mature messenger RNA derived from the gene, but is not necessarily a part of the sequence that encodes the final gene product.

The "translation leader sequence" refers to a DNA sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is 15 present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect processing of the primary transcript to mRNA, mRNA stability or translation efficiency. Examples of translation leader sequences have been described (Turner, R. and Foster, 20 G. D. (1995) *Molecular Biotechnology* 3:225).

The "3' non-coding sequences" refer to DNA sequences located downstream of a coding sequence and include polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA 25 processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht et al., (1989) *Plant Cell* 1:671-680.

30 "RNA transcript" refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from post-transcriptional 35 processing of the primary transcript and is referred to as the

mature RNA. "Messenger RNA (mRNA)" refers to the RNA that is without introns and that can be translated into protein by the cell. "cDNA" refers to a DNA that is complementary to and synthesized from a mRNA template using the enzyme reverse transcriptase. The cDNA can be single-stranded or converted into the double-stranded form using the Klenow fragment of DNA polymerase I. "Sense" RNA refers to RNA transcript that includes the mRNA and can be translated into protein within a cell or *in vitro*. "Antisense RNA" refers to an RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene (U.S. Patent No. 5,107,065). The complementarity of an antisense RNA may be with any part of the specific gene transcript, i.e., at the 5' non-coding sequence, 3' non-coding sequence, introns, or the coding sequence. "Functional RNA" refers to antisense RNA, ribozyme RNA, or other RNA that may not be translated but yet has an effect on cellular processes. The terms "complement" and "reverse complement" are used interchangeably herein with respect to mRNA transcripts, and are meant to define the antisense RNA of the message.

The term "endogenous RNA" refers to any RNA which is encoded by any nucleic acid sequence present in the genome of the host prior to transformation with the recombinant construct of the present invention, whether naturally-occurring or non-naturally occurring, i.e., introduced by recombinant means, mutagenesis, etc.

The term "non-naturally occurring" means artificial, not consistent with what is normally found in nature.

The term "operably linked" refers to the association of nucleic acid sequences on a single nucleic acid fragment so that the function of one is regulated by the other. For example, a promoter is operably linked with a coding sequence when it is capable of regulating the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences

can be operably linked to regulatory sequences in a sense or antisense orientation. In another example, the complementary RNA regions of the invention can be operably linked, either directly or indirectly, 5' to the target mRNA, or 3' to the target mRNA, or within the target mRNA, or a first complementary region is 5' and its complement is 3' to the target mRNA.

The term "expression", as used herein, refers to the production of a functional end-product. Expression of a gene involves transcription of the gene and translation of the mRNA into a precursor or mature protein. "Antisense inhibition" refers to the production of antisense RNA transcripts capable of suppressing the expression of the target protein. "Co-suppression" refers to the production of sense RNA transcripts capable of suppressing the expression of identical or substantially similar foreign or endogenous genes (U.S. Patent No. 5,231,020).

"Mature" protein refers to a post-translationally processed polypeptide; i.e., one from which any pre- or propeptides present in the primary translation product have been removed. "Precursor" protein refers to the primary product of translation of mRNA; i.e., with pre- and pro-peptides still present. Pre- and pro-peptides may be but are not limited to intracellular localization signals.

"Stable transformation" refers to the transfer of a nucleic acid fragment into a genome of a host organism, resulting in genetically stable inheritance. In contrast, "transient transformation" refers to the transfer of a nucleic acid fragment into the nucleus, or DNA-containing organelle, of a host organism resulting in gene expression without integration or stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" organisms. The preferred method of cell transformation of rice, corn and other monocots is the use of particle-accelerated or "gene gun" transformation technology

(Klein et al., (1987) *Nature (London)* 327:70-73; U.S. Patent No. 4,945,050), or an *Agrobacterium*-mediated method using an appropriate Ti plasmid containing the transgene (Ishida Y. et al., 1996, *Nature Biotech.* 14:745-750). The term 5 "transformation" as used herein refers to both stable transformation and transient transformation.

Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook, J., Fritsch, E.F. and Maniatis, T. 10 *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989 (hereinafter "Sambrook").

The term "recombinant" refers to an artificial combination of two otherwise separated segments of sequence, 15 e.g., by chemical synthesis or by the manipulation of isolated segments of nucleic acids by genetic engineering techniques.

"PCR" or "Polymerase Chain Reaction" is a technique for the synthesis of large quantities of specific DNA segments, consists of a series of repetitive cycles (Perkin Elmer Cetus 20 Instruments, Norwalk, CT). Typically, the double stranded DNA is heat denatured, the two primers complementary to the 3' boundaries of the target segment are annealed at low temperature and then extended at an intermediate temperature. One set of these three consecutive steps is referred to as a 25 cycle.

Polymerase chain reaction ("PCR") is a powerful technique used to amplify DNA millions of fold, by repeated replication of a template, in a short period of time. (Mullis et al, *Cold Spring Harbor Symp. Quant. Biol.* 51:263-273 (1986); Erlich 30 et al, European Patent Application 50,424; European Patent Application 84,796; European Patent Application 258,017, European Patent Application 237,362; Mullis, European Patent Application 201,184, Mullis et al U.S. Patent No. 4,683,202; Erlich, U.S. Patent No. 4,582,788; and Saiki et al, U.S. 35 Patent No. 4,683,194). The process utilizes sets of specific

in vitro synthesized oligonucleotides to prime DNA synthesis. The design of the primers is dependent upon the sequences of DNA that are desired to be analyzed. The technique is carried out through many cycles (usually 20-50) of melting the 5 template at high temperature, allowing the primers to anneal to complementary sequences within the template and then replicating the template with DNA polymerase.

The products of PCR reactions are analyzed by separation in agarose gels followed by ethidium bromide staining and 10 visualization with UV transillumination. Alternatively, radioactive dNTPs can be added to the PCR in order to incorporate label into the products. In this case the products of PCR are visualized by exposure of the gel to x-ray film. The added advantage of radiolabeling PCR products is 15 that the levels of individual amplification products can be quantitated.

The terms "recombinant construct", "expression construct" and "recombinant expression construct" are used interchangeably herein. These terms refer to a functional unit 20 of genetic material that can be inserted into the genome of a cell using standard methodology well known to one skilled in the art. Such construct may be itself or may be used in conjunction with a vector. If a vector is used then the choice of vector is dependent upon the method that will be 25 used to transform host plants as is well known to those skilled in the art. For example, a plasmid vector can be used. The skilled artisan is well aware of the genetic elements that must be present on the vector in order to successfully transform, select and propagate host cells 30 comprising any of the isolated nucleic acid fragments of the invention. The skilled artisan will also recognize that different independent transformation events will result in different levels and patterns of expression (Jones et al., (1985) *EMBO J.* 4:2411-2418; De Almeida et al., (1989) *Mol. 35 Gen. Genetics* 218:78-86), and thus that multiple events must

be screened in order to obtain lines displaying the desired expression level and pattern. Such screening may be accomplished by Southern analysis of DNA, Northern analysis of mRNA expression, Western analysis of protein expression, or 5 phenotypic analysis.

Production of the Δ4-Desaturase Enzyme

Once the gene encoding the Δ4-desaturase enzyme has been isolated, it may then be introduced into either a prokaryotic 10 or eukaryotic host cell through the use of a vector or construct. The vector, for example, a bacteriophage, cosmid or plasmid, may comprise the nucleotide sequence encoding the Δ4-desaturase enzyme, as well as any regulatory sequence (e.g., promoter) which is functional in the host cell and is 15 able to elicit expression of the desaturase encoded by the nucleotide sequence. The regulatory sequence (e.g., promoter) is in operable association with or operably linked to the nucleotide sequence. (A promoter is said to be "operably linked" with a coding sequence if the promoter affects 20 transcription or expression of the coding sequence.) Suitable promoters include, for example, those from genes encoding alcohol dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglucoisomerase, phosphoglycerate kinase, acid phosphatase, T7, TPI, lactase, metallothionein, 25 cytomegalovirus immediate early, whey acidic protein, glucoamylase, and promoters activated in the presence of galactose, for example, GAL1 and GAL10. Additionally, nucleotide sequences which encode other proteins, oligosaccharides, lipids, etc. may also be included within the 30 vector as well as other regulatory sequences such as a polyadenylation signal (e.g., the poly-A signal of SV-40T- antigen, ovalbumin or bovine growth hormone). The choice of sequences present in the construct is dependent upon the desired

expression products as well as the nature of the host cell.

As noted above, once the vector has been constructed, it may then be introduced into the host cell of choice by methods known to those of ordinary skill in the art including, for 5 example, transfection, transformation and electroporation (see Molecular Cloning: A Laboratory Manual, 2<sup>nd</sup> ed., Vol. 1-3, ed. Sambrook et al., Cold Spring Harbor Laboratory Press (1989)). The host cell is then cultured under suitable conditions permitting expression of the genes leading to the production 10 of the desired PUFA, which is then recovered and purified.

Examples of suitable prokaryotic host cells include, for example, bacteria such as Escherichia coli, Bacillus subtilis as well as Cyanobacteria such as Spirulina spp. (i.e., blue-green algae). Examples of suitable eukaryotic host cells 15 include, for example, mammalian cells, plant cells, yeast cells such as Saccharomyces cerevisiae, Saccharomyces carlsbergensis, Lipomyces starkey, Candida spp. such as Yarrowia (Candida) lipolytica, Kluyveromyces spp., Pichia spp., Trichoderma spp. or Hansenula spp., or fungal cells such 20 as filamentous fungal cells, for example, Aspergillus, Neurospora and Penicillium. Preferably, Saccharomyces cerevisiae (baker's yeast) cells are utilized.

Expression in a host cell can be accomplished in a transient or stable fashion. Transient expression can occur 25 from introduced constructs which contain expression signals functional in the host cell, but which constructs do not replicate and rarely integrate in the host cell, or when the host cell is not proliferating. Transient expression also can be accomplished by inducing the activity of a regulatable promoter operably linked to the gene of interest, although such inducible systems frequently exhibit a low basal level of expression. Stable expression can be achieved by introduction of a construct that can integrate into the host genome or that autonomously replicates in the host cell. Stable expression 30 of the gene of interest can be selected through the use of a 35

selectable marker located on or transfected with the expression construct, followed by selection for cells expressing the marker. When stable expression results from integration, the site of the construct's integration can occur 5 randomly within the host genome or can be targeted through the use of constructs containing regions of homology with the host genome sufficient to target recombination with the host locus. Where constructs are targeted to an endogenous locus, all or some of the transcriptional and translational regulatory 10 regions can be provided by the endogenous locus.

A transgenic mammal may also be used in order to express the enzyme of interest (i.e.,  $\Delta$ 4-desaturase), and ultimately the PUFA(s) of interest. More specifically, once the above-described construct is created, it may be inserted into the 15 pronucleus of an embryo. The embryo may then be implanted into a recipient female. Alternatively, a nuclear transfer method could also be utilized (Schnieke et al., Science 278:2130-2133 (1997)). Gestation and birth are then permitted (see, e.g., U.S. Patent No. 5,750,176 and U.S. Patent No. 20 5,700,671). Milk, tissue or other fluid samples from the offspring should then contain altered levels of PUFAs, as compared to the levels normally found in the non-transgenic animal. Subsequent generations may be monitored for 25 production of the altered or enhanced levels of PUFAs and thus incorporation of the gene encoding the desired desaturase enzyme into their genomes. The mammal utilized as the host may be selected from the group consisting of, for example, a mouse, a rat, a rabbit, a pig, a goat, a sheep, a horse and a cow. However, any mammal may be used provided it has the 30 ability to incorporate DNA encoding the enzyme of interest into its genome.

For expression of a desaturase polypeptide, functional transcriptional and translational initiation and termination regions are operably linked to the DNA encoding the desaturase 35 polypeptide. Transcriptional and translational initiation and

termination regions are derived from a variety of nonexclusive sources, including the DNA to be expressed, genes known or suspected to be capable of expression in the desired system, expression vectors, chemical synthesis, or from an endogenous 5 locus in a host cell. Expression in a plant tissue and/or plant part presents certain efficiencies, particularly where the tissue or part is one which is harvested early, such as seed, leaves, fruits, flowers, roots, etc. Expression can be targeted to that location with the plant by utilizing specific 10 regulatory sequence such as those of U.S. Patent Nos. 5,463,174, 4,943,674, 5,106,739, 5,175,095, 5,420,034, 5,188,958, and 5,589,379. Alternatively, the expressed 15 protein can be an enzyme which produces a product which may be incorporated, either directly or upon further modifications, into a fluid fraction from the host plant. Expression of a desaturase gene, or antisense desaturase transcripts, can alter the levels of specific PUFAs, or derivatives thereof, found in plant parts and/or plant tissues. The desaturase polypeptide coding region may be expressed either by itself or 20 with other genes, in order to produce tissues and/or plant parts containing higher proportions of desired PUFAs or in which the PUFA composition more closely resembles that of human breast milk (Prieto et al., PCT publication WO 95/24494). The termination region may be derived from the 3' 25 region of the gene from which the initiation region was obtained or from a different gene. A large number of termination regions are known to and have been found to be satisfactory in a variety of hosts from the same and different genera and species. The termination region usually is 30 selected as a matter of convenience rather than because of any particular property.

As noted above, a plant (e.g., Glycine max (soybean) or Brassica napus (canola)) or plant tissue may also be utilized as a host or host cell, respectively, for expression of the 35 desaturase enzyme which may, in turn, be utilized in the

production of polyunsaturated fatty acids. More specifically, desired PUFAS can be expressed in seed. Methods of isolating seed oils are known in the art. Thus, in addition to providing a source for PUFAs, seed oil components may be manipulated through the expression of the desaturase gene, as well as perhaps other desaturase genes and elongase genes, in order to provide seed oils that can be added to nutritional compositions, pharmaceutical compositions, animal feeds and cosmetics. Once again, a vector which comprises a DNA sequence encoding the desaturase operably linked to a promoter, will be introduced into the plant tissue or plant for a time and under conditions sufficient for expression of the desaturase gene. The vector may also comprise one or more genes that encode other enzymes, for example,  $\Delta 5$ -desaturase, elongase,  $\Delta 12$ -desaturase,  $\Delta 15$ -desaturase,  $\Delta 17$ -desaturase, and/or  $\Delta 19$ -desaturase. The plant tissue or plant may produce the relevant substrate (e.g., adrenic acid or  $\omega 3$ -docosapentaenoic acid) upon which the enzyme acts or a vector encoding enzymes which produce such substrates may be introduced into the plant tissue, plant cell or plant. In addition, substrate may be sprayed on plant tissues expressing the appropriate enzymes. Using these various techniques, one may produce PUFAs (e.g., n-6 unsaturated fatty acids such as  $\omega 6$ -docosapentaenoic acid, or n-3 fatty acids such as docosahexaenoic acid) by use of a plant cell, plant tissue or plant. It should also be noted that the invention also encompasses a transgenic plant comprising the above-described vector, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid in, for example, the seeds of the transgenic plant.

The regeneration, development, and cultivation of plants from single plant protoplast transformants or from various transformed explants is well known in the art (Weissbach and Weissbach, In: Methods for Plant Molecular Biology, (Eds.), Academic Press, Inc. San Diego, CA, (1988)).

This regeneration and growth process typically includes the steps of selection of transformed cells, culturing those individualized cells through the usual stages of embryonic development through the rooted plantlet stage. Transgenic 5 embryos and seeds are similarly regenerated. The resulting transgenic rooted shoots are thereafter planted in an appropriate plant growth medium such as soil.

The development or regeneration of plants containing the foreign, exogenous gene that encodes a protein of interest is 10 well known in the art. Preferably, the regenerated plants are self-pollinated to provide homozygous transgenic plants. Otherwise, pollen obtained from the regenerated plants is crossed to seed-grown plants of agronomically important lines. Conversely, pollen from plants of these important lines is 15 used to pollinate regenerated plants. A transgenic plant of the present invention containing a desired polypeptide is cultivated using methods well known to one skilled in the art.

There are a variety of methods for the regeneration of plants from plant tissue. The particular method of 20 regeneration will depend on the starting plant tissue and the particular plant species to be regenerated.

Methods for transforming dicots, primarily by use of *Agrobacterium tumefaciens*, and obtaining transgenic plants have been published for cotton (U.S. Patent No. 5,004,863, 25 U.S. Patent No. 5,159,135, U.S. Patent No. 5,518,908); soybean (U.S. Patent No. 5,569,834, U.S. Patent No. 5,416,011, McCabe et. al., BiolTechnology 6:923 (1988), Christou et al., Plant Physiol. 87:671-674 (1988)); Brassica (U.S. Patent No. 5,463,174); peanut (Cheng et al., Plant Cell Rep. 30 15:653-657 (1996), McKently et al., Plant Cell Rep. 14:699-703 (1995)); papaya; and pea (Grant et al., Plant Cell Rep. 15:254-258, (1995)).

Transformation of monocotyledons using electroporation, particle bombardment, and *Agrobacterium* have also been 35 reported. Transformation and plant regeneration have been

achieved in asparagus (Bytebier et al., Proc. Natl. Acad. Sci. (USA) 84:5354, (1987)); barley (Wan and Lemaux, Plant Physiol 104:37 (1994)); Zea mays (Rhodes et al., Science 240:204 (1988), Gordon-Kamm et al., Plant Cell 2:603-618 (1990), Fromm et al., BiolTechnology 8:833 (1990), Koziel et al., BiolTechnology 11:194, (1993), Armstrong et al., Crop Science 35:550-557 (1995)); oat (Somers et al., BiolTechnology 10:1589 (1992)); orchard grass (Horn et al., Plant Cell Rep. 7:469 (1988)); rice (Toriyama et al., TheorAppl. Genet. 205:34 (1986); Part et al., Plant Mol. Biol. 32:1135-1148, (1996); Abedinia et al., Aust. J. Plant Physiol. 24:133-141 (1997); Zhang and Wu, Theor. Appl. Genet. 76:835 (1988); Zhang et al. Plant Cell Rep. 7:379, (1988); Battraw and Hall, Plant Sci. 86:191-202 (1992); Christou et al., Bio/Technology 9:957 (1991)); rye (De la Pena et al., Nature 325:274 (1987)); sugarcane (Bower and Birch, Plant J. 2:409 (1992)); tall fescue (Wang et al., BiolTechnology 10:691 (1992)), and wheat (Vasil et al., BiolTechnology 10:667 (1992); U.S. Patent No. 5,631,152).

Assays for gene expression based on the transient expression of cloned nucleic acid constructs have been developed by introducing the nucleic acid molecules into plant cells by polyethylene glycol treatment, electroporation, or particle bombardment (Marcotte et al., Nature 335:454-457 (1988); Marcotte et al., Plant Cell 1:523-532 (1989); McCarty et al., Cell 66:895-905 (1991); Hattori et al., Genes Dev. 6:609-618 (1992); Goff et al., EMBO J. 9:2517-2522 (1990)).

Transient expression systems may be used to functionally dissect gene constructs (see generally, Maliga et al., Methods in Plant Molecular Biology, Cold Spring Harbor Press (1995)). It is understood that any of the nucleic acid molecules of the present invention can be introduced into a plant cell in a permanent or transient manner in combination with other genetic elements such as vectors, promoters, enhancers etc.

In addition to the above discussed procedures, practitioners are familiar with the standard resource materials which describe specific conditions and procedures for the construction, manipulation and isolation of

5 macromolecules (e.g., DNA molecules, plasmids, etc.), generation of recombinant organisms and the screening and isolating of clones, (see for example, Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (1989); Maliga et al., Methods in Plant Molecular

10 Biology, Cold Spring Harbor Press (1995); Birren et al., Genome Analysis: Detecting Genes, 1, Cold Spring Harbor, New York (1998); Birren et al., Genome Analysis: Analyzing DNA, 2, Cold Spring Harbor, New York (1998); Plant Molecular Biology: A Laboratory Manual, eds. Clark, Springer, New York (1997)).

15 The substrates which may be produced by the host cell either naturally or transgenically, as well as the enzymes which may be encoded by DNA sequences present in the vector which is subsequently introduced into the host cell, are shown in Figure 1.

20

Uses of the  $\Delta 4$ -Desaturase Gene and Enzyme Encoded Thereby

As noted above, the isolated desaturase genes and the desaturase enzymes encoded thereby have many uses. For example, the gene and corresponding enzyme may be used

25 indirectly or directly in the production of polyunsaturated fatty acids, for example,  $\Delta 4$ -desaturase may be used in the production of  $\omega 6$ -docosapentaenoic acid or docosahexaenoic acid. "Directly" is meant to encompass the situation where the enzyme directly converts the acid to another acid, the latter

30 of which is utilized in a composition (e.g., the conversion of adrenic acid to  $\omega 6$ -docosapentaenoic acid). "Indirectly" is meant to encompass the situation where an acid is converted to another acid (i.e., a pathway intermediate) by the desaturase (e.g., adrenic acid to  $\omega 6$ -docosapentaenoic acid) and then the

35 latter acid is converted to another acid by use of a

desaturase or non-desaturase enzyme (e.g.,  $\omega 6$ -docosapentaenoic acid to docosahexaenoic acid by  $\Delta 19$ -desaturase). Also, the present invention includes "indirect" situations in which the PUFA is first converted to another polyunsaturated fatty acid 5 by a non- $\Delta 4$ -desaturase enzyme (for example, an elongase or another desaturase) and then converted to a final product via  $\Delta 4$ -desaturase. For example, eicosapentaenoic acid may be converted to  $\omega 3$ -docosapentaenoic acid by an elongase, and then converted to docosahexaenoic acid by a  $\Delta 4$ -desaturase. These 10 polyunsaturated fatty acids (i.e., those produced either directly or indirectly by activity of the desaturase enzyme) may be added to, for example, nutritional compositions, pharmaceutical compositions, cosmetics, and animal feeds, all of which are encompassed by the present invention. These uses 15 are described, in detail, below.

#### Nutritional Compositions

The present invention includes nutritional compositions. Such compositions, for purposes of the present invention, 20 include any food or preparation for human consumption including for enteral or parenteral consumption, which when taken into the body (a) serve to nourish or build up tissues or supply energy and/or (b) maintain, restore or support adequate nutritional status or metabolic function.

25 The nutritional composition of the present invention comprises at least one oil or acid produced directly or indirectly by use of the desaturase gene, in accordance with the present invention, and may either be in a solid or liquid form. Additionally, the composition may include edible 30 macronutrients, vitamins and minerals in amounts desired for a particular use. The amount of such ingredients will vary depending on whether the composition is intended for use with normal, healthy infants, children or adults having specialized needs such as those which accompany certain metabolic 35 conditions (e.g., metabolic disorders).

Examples of macronutrients which may be added to the composition include but are not limited to edible fats, carbohydrates and proteins. Examples of such edible fats include but are not limited to coconut oil, borage oil, fungal oil, black current oil, soy oil, and mono- and diglycerides. Examples of such carbohydrates include but are not limited to glucose, edible lactose and hydrolyzed starch. Additionally, examples of proteins which may be utilized in the nutritional composition of the invention include but are not limited to 5 soy proteins, electrodialysed whey, electrodialysed skim milk, milk whey, or the hydrolysates of these proteins. 10

With respect to vitamins and minerals, the following may be added to the nutritional compositions of the present invention: calcium, phosphorus, potassium, sodium, chloride, 15 magnesium, manganese, iron, copper, zinc, selenium, iodine, and Vitamins A, E, D, C, and the B complex. Other such vitamins and minerals may also be added.

The components utilized in the nutritional compositions of the present invention will be of semi-purified or purified 20 origin. By semi-purified or purified is meant a material which has been prepared by purification of a natural material or by synthesis.

Examples of nutritional compositions of the present invention include but are not limited to infant formulas, 25 dietary supplements (e.g., adult nutritional products and oil), dietary substitutes, and rehydration compositions. Nutritional compositions of particular interest include but are not limited to those utilized for enteral and parenteral supplementation for infants, specialist infant formulas, 30 supplements for the elderly, and supplements for those with gastrointestinal difficulties and/or malabsorption.

The nutritional composition of the present invention may also be added to food even when supplementation of the diet is not required. For example, the composition may be added to 35 food of any type including but not limited to margarines,

modified butters, cheeses, milk, yogurt, chocolate, candy, snacks, salad oils, cooking oils, cooking fats, meats, fish and beverages.

In a preferred embodiment of the present invention, the 5 nutritional composition is an enteral nutritional product, more preferably, an adult or pediatric enteral nutritional product. This composition may be administered to adults or children experiencing stress or having specialized needs due to chronic or acute disease states. The composition may 10 comprise, in addition to polyunsaturated fatty acids produced in accordance with the present invention, macronutrients, vitamins and minerals as described above. The macronutrients may be present in amounts equivalent to those present in human milk or on an energy basis, i.e., on a per calorie basis.

15 Methods for formulating liquid or solid enteral and parenteral nutritional formulas are well known in the art. (See also the Examples below.)

The enteral formula, for example, may be sterilized and subsequently utilized on a ready-to-feed (RTF) basis or stored 20 in a concentrated liquid or powder. The powder can be prepared by spray drying the formula prepared as indicated above, and reconstituting it by rehydrating the concentrate. Adult and pediatric nutritional formulas are well known in the art and are commercially available (e.g., Similac®, Ensure®, 25 Jevity® and Alimentum® from Ross Products Division, Abbott Laboratories, Columbus, Ohio). An oil or acid produced in accordance with the present invention may be added to any of these formulas.

The energy density of the nutritional compositions of the 30 present invention, when in liquid form, may range from about 0.6 Kcal to about 3 Kcal per ml. When in solid or powdered form, the nutritional supplements may contain from about 1.2 to more than 9 Kcals per gram, preferably about 3 to 7 Kcals per gm. In general, the osmolality of a liquid product should

be less than 700 mOsm and, more preferably, less than 660 mOsm.

The nutritional formula may include macronutrients, vitamins, and minerals, as noted above, in addition to the 5 PUFAs produced in accordance with the present invention. The presence of these additional components helps the individual ingest the minimum daily requirements of these elements. In addition to the provision of PUFAs, it may also be desirable to add zinc, copper, folic acid and antioxidants to the 10 composition. It is believed that these substances boost a stressed immune system and will therefore provide further benefits to the individual receiving the composition. A pharmaceutical composition may also be supplemented with these 15 elements.

15 In a more preferred embodiment, the nutritional composition comprises, in addition to antioxidants and at least one PUFA, a source of carbohydrate wherein at least 5 weight percent of the carbohydrate is indigestible oligosaccharide. In a more preferred embodiment, the 20 nutritional composition additionally comprises protein, taurine, and carnitine.

As noted above, the PUFAs produced in accordance with the present invention, or derivatives thereof, may be added to a dietary substitute or supplement, particularly an infant 25 formula, for patients undergoing intravenous feeding or for preventing or treating malnutrition or other conditions or disease states. As background, it should be noted that human breast milk has a fatty acid profile comprising from about 0.15% to about 0.36% as DHA, from about 0.03% to about 0.13% 30 as EPA, from about 0.30% to about 0.88% as AA, from about 0.22% to about 0.67% as DGLA, and from about 0.27% to about 1.04% as GLA. Thus, fatty acids such as AA, EPA and/or 35 docosahexaenoic acid (DHA), produced in accordance with the present invention, can be used to alter, for example, the composition of infant formulas in order to better replicate

the PUFA content of human breast milk or to alter the presence of PUFAs normally found in a non-human mammal's milk. In particular, a composition for use in a pharmacologic or food supplement, particularly a breast milk substitute or supplement, will preferably comprise one or more of AA, DGLA and GLA. More preferably, the oil will comprise from about 0.3 to 30% AA, from about 0.2 to 30% DGLA, and/or from about 0.2 to about 30% GLA.

Parenteral nutritional compositions comprising from about 10 2 to about 30 weight percent fatty acids calculated as triglycerides are encompassed by the present invention. The preferred composition has about 1 to about 25 weight percent of the total PUFA composition as GLA (U.S. Patent No. 5,196,198). Other vitamins, particularly fat-soluble vitamins 15 such as vitamin A, D, E and L-carnitine can optionally be included. When desired, a preservative such as alpha-tocopherol may be added in an amount of about 0.1% by weight.

In addition, the ratios of AA, DGLA and GLA can be adapted for a particular given end use. When formulated as a 20 breast milk supplement or substitute, a composition which comprises one or more of AA, DGLA and GLA will be provided in a ratio of about 1:19:30 to about 6:1:0.2, respectively. For example, the breast milk of animals can vary in ratios of AA:DGLA:GLA ranging from 1:19:30 to 6:1:0.2, which includes 25 intermediate ratios which are preferably about 1:1:1, 1:2:1, 1:1:4. When produced together in a host cell, adjusting the rate and percent of conversion of a precursor substrate such as GLA and DGLA to AA can be used to precisely control the PUFA ratios. For example, a 5% to 10% conversion rate of DGLA 30 to AA can be used to produce an AA to DGLA ratio of about 1:19, whereas a conversion rate of about 75% TO 80% can be used to produce an AA to DGLA ratio of about 6:1. Therefore, whether in a cell culture system or in a host animal, regulating the timing, extent and specificity of desaturase 35 expression, as well as the expression of other desaturases and

elongases, can be used to modulate PUFA levels and ratios. The PUFAs produced in accordance with the present invention (e.g., AA and EPA) may then be combined with other PUFAs/acids (e.g., GLA) in the desired concentrations and ratios.

5        Additionally, PUFA produced in accordance with the present invention or host cells containing them may also be used as animal food supplements to alter an animal's tissue or milk fatty acid composition to one more desirable for human or animal consumption.

10

#### Pharmaceutical Compositions

The present invention also encompasses a pharmaceutical composition comprising one or more of the acids and/or resulting oils produced using the desaturase genes, in accordance with the methods described herein. More specifically, such a pharmaceutical composition may comprise one or more of the acids and/or oils as well as a standard, well-known, non-toxic pharmaceutically acceptable carrier, adjuvant or vehicle such as, for example, phosphate buffered saline, water, ethanol, polyols, vegetable oils, a wetting agent or an emulsion such as a water/oil emulsion. The composition may be in either a liquid or solid form. For example, the composition may be in the form of a tablet, capsule, ingestible liquid or powder, injectible, or topical ointment or cream. Proper fluidity can be maintained, for example, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants. It may also be desirable to include isotonic agents, for example, sugars, sodium chloride and the like. Besides such inert diluents, the composition can also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening agents, flavoring agents and perfuming agents.

Suspensions, in addition to the active compounds, may comprise suspending agents such as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan

esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth or mixtures of these substances.

Solid dosage forms such as tablets and capsules can be 5 prepared using techniques well known in the art. For example, PUFAs produced in accordance with the present invention can be tableted with conventional tablet bases such as lactose, sucrose, and cornstarch in combination with binders such as acacia, cornstarch or gelatin, disintegrating agents such as 10 potato starch or alginic acid, and a lubricant such as stearic acid or magnesium stearate. Capsules can be prepared by incorporating these excipients into a gelatin capsule along with antioxidants and the relevant PUFA(s). The antioxidant and PUFA components should fit within the guidelines presented 15 above.

For intravenous administration, the PUFAs produced in accordance with the present invention or derivatives thereof may be incorporated into commercial formulations such as Intralipids™. The typical normal adult plasma fatty acid 20 profile comprises 6.64 to 9.46% of AA, 1.45 to 3.11% of DGLA, and 0.02 to 0.08% of GLA. These PUFAs or their metabolic precursors can be administered alone or in combination with other PUFAs in order to achieve a normal fatty acid profile in a patient. Where desired, the individual components of the 25 formulations may be provided individually, in kit form, for single or multiple use. A typical dosage of a particular fatty acid is from 0.1 mg to 20 g (up to 100 g) daily and is preferably from 10 mg to 1, 2, 5 or 10 g daily.

Possible routes of administration of the pharmaceutical 30 compositions of the present invention include, for example, enteral (e.g., oral and rectal) and parenteral. For example, a liquid preparation may be administered, for example, orally or rectally. Additionally, a homogenous mixture can be completely dispersed in water, admixed under sterile 35 conditions with physiologically acceptable diluents,

preservatives, buffers or propellants in order to form a spray or inhalant.

The route of administration will, of course, depend upon the desired effect. For example, if the composition is being

5 utilized to treat rough, dry, or aging skin, to treat injured or burned skin, or to treat skin or hair affected by a disease or condition, it may perhaps be applied topically.

The dosage of the composition to be administered to the patient may be determined by one of ordinary skill in the art 10 and depends upon various factors such as weight of the patient, age of the patient, immune status of the patient, etc.

With respect to form, the composition may be, for example, a solution, a dispersion, a suspension, an emulsion 15 or a sterile powder which is then reconstituted.

The present invention also includes the treatment of various disorders by use of the pharmaceutical and/or nutritional compositions described herein. In particular, the compositions of the present invention may be used to treat 20 restenosis after angioplasty. Furthermore, symptoms of inflammation, rheumatoid arthritis, asthma and psoriasis may also be treated with the compositions of the invention.

Evidence also indicates that PUFAs may be involved in calcium metabolism; thus, the compositions of the present invention 25 may, perhaps, be utilized in the treatment or prevention of osteoporosis and of kidney or urinary tract stones.

Additionally, the compositions of the present invention may also be used in the treatment of cancer. Malignant cells have been shown to have altered fatty acid compositions. 30 Addition of fatty acids has been shown to slow their growth, cause cell death and increase their susceptibility to chemotherapeutic agents. Moreover, the compositions of the present invention may also be useful for treating cachexia associated with cancer.

The compositions of the present invention may also be used to treat diabetes (see U.S. Patent No. 4,826,877 and Horrobin et al., Am. J. Clin. Nutr. Vol. 57 (Suppl.) 732S-737S). Altered fatty acid metabolism and composition have 5 been demonstrated in diabetic animals.

Furthermore, the compositions of the present invention, comprising PUFAs produced either directly or indirectly through the use of the desaturase enzymes, may also be used in the treatment of eczema, in the reduction of blood pressure, 10 and in the improvement of mathematics examination scores. Additionally, the compositions of the present invention may be used in inhibition of platelet aggregation, induction of vasodilation, reduction in cholesterol levels, inhibition of proliferation of vessel wall smooth muscle and fibrous tissue 15 (Brenner et al., Adv. Exp. Med. Biol. Vol. 83, p.85-101, 1976), reduction or prevention of gastrointestinal bleeding and other side effects of non-steroidal anti-inflammatory drugs (see U.S. Patent No. 4,666,701), prevention or treatment of endometriosis and premenstrual syndrome (see U.S. Patent 20 No. 4,758,592), and treatment of myalgic encephalomyelitis and chronic fatigue after viral infections (see U.S. Patent No. 5,116,871).

Further uses of the compositions of the present invention include use in the treatment of AIDS, multiple sclerosis, and 25 inflammatory skin disorders, as well as for maintenance of general health.

Additionally, the composition of the present invention may be utilized for cosmetic purposes. It may be added to pre-existing cosmetic compositions such that a mixture is 30 formed or may be used as a sole composition.

#### Veterinary Applications

It should be noted that the above-described pharmaceutical and nutritional compositions may be utilized in 35 connection with animals (i.e., domestic or non-domestic), as

well as humans, as animals experience many of the same needs and conditions as humans. For example, the oil or acids of the present invention may be utilized in animal feed supplements, animal feed substitutes, animal vitamins or in 5 animal topical ointments.

The present invention may be illustrated by the use of the following non-limiting examples:

Example I

10 Design of Degenerate Oligonucleotides for the Isolation of Desaturases from *Thraustochytrium aureum* and cDNA Library Construction

15 The fatty acid composition analysis of the marine fungus *Thraustochytrium aureum* (*T. aureum*) (ATCC 34304) was investigated to determine the types and amounts of polyunsaturated fatty acids (PUFAs). This fungus had substantial amounts of longer chain PUFAs such as arachidonic acid (ARA, 20:4n-6) and eicosapentaenoic acid (EPA, 20:5 n-3). 20 However, *T. aureum* also produced PUFAs such as adrenic acid (ADA, 22:4n-6),  $\omega$ 6-docosapentaenoic acid ( $\omega$ 6-DPA, 22:5n-6),  $\omega$ 3-docosapentaenoic acid ( $\omega$ 3-DPA, 22:5n-3), with the highest amount of fatty acid being docosahexaenoic acid (DHA, 22:6n-3) (see Figure 1). Thus in addition to  $\Delta$ 6-,  $\Delta$ 5- and  $\Delta$ 17- 25 desaturases, *T. aureum* probably contains a  $\Delta$ 19-desaturase which converts ADA to  $\omega$ 3-DPA or  $\omega$ 6-DPA to DHA and/or a  $\Delta$ 4-desaturase which desaturates ADA to  $\omega$ 6-DPA or  $\omega$ 3-DPA to DHA. The goal was therefore to attempt to isolate the predicted 30 desaturase genes from *T. aureum*, and to verify the functionality of the enzymes by expression in an alternate host.

To isolate genes encoding for functional desaturase enzymes, a cDNA library was constructed. *T. aureum* (ATCC 34304) cells were grown in BY+ Media (#790, Difco, Detroit, MI) at room temperature for 4 days, in the presence of light, and with constant agitation (250 rpm) to obtain the maximum biomass. These cells were harvested by centrifugation at 5000 rpm for 10 minutes and rinsed in ice-cold RNase-free water.

These cells were then lysed in a French press at 10,000 psi, and the lysed cells were directly collected into TE buffered phenol. Proteins from the cell lysate were removed by repeated phenol: chloroform (1:1 v/v) extraction, followed by 5 a chloroform extraction. The nucleic acids from the aqueous phase were precipitated at -70°C for 30 minutes using 0.3M (final concentration) sodium acetate (pH 5.6) and one volume of isopropanol. The precipitated nucleic acids were collected by centrifugation at 15,000 rpm for 30 minutes at 4°C, vacuum- 10 dried for 5 minutes and then treated with DNaseI (RNase-free) in 1X DNase buffer (20 mM Tris-Cl, pH 8.0; 5mM MgCl<sub>2</sub>) for 15 minutes at room temperature. The reaction was quenched with 5 mM EDTA (pH 8.0) and the RNA further purified using the Qiagen RNeasy Maxi kit (Qiagen, Valencia, CA) as per the 15 manufacturer's protocol.

Messenger RNA was isolated from total RNA using oligo dT cellulose resin, and the pBluescript II XR library construction kit (Stratagene, La Jolla, CA) was used to synthesize double stranded cDNA which was then directionally 20 cloned (5' EcoRI/3' XhoI) into pBluescript II SK(+) vector (Stratagene, La Jolla, CA). The *T. aureum* library contained approximately 2.5 x 10<sup>6</sup> clones each with an average insert size of approximately 700 bp. Genomic DNA from PUFA-producing *T. aureum* cultures was isolated by crushing the culture in liquid 25 nitrogen and was purified using Qiagen Genomic DNA Extraction Kit (Qiagen, Valencia, CA).

The approach taken was to design degenerate oligonucleotides (primers) that represent amino acid motifs that are conserved in known desaturases. These primers could 30 be then used in a PCR reaction to identify a fragment containing the conserved regions in the predicted desaturase genes from fungi. Since the only fungal desaturases which have been identified are  $\Delta$ 5- and  $\Delta$ 6-desaturase genes from *Mortierella alpina* (Genbank accession numbers AF067650, 35 AB020032, respectively), desaturase sequences from plants as well as animals were taken into consideration during the design of these degenerate primers. In particular, known  $\Delta$ 5- and  $\Delta$ 6-desaturase sequences from the following organisms were

used for the design of these degenerate primers: *Mortierella alpina*, *Borago officinalis*, *Helianthus annuus*, *Brassica napus*, *Dictyostelium discoideum*, *Rattus norvegicus*, *Mus musculus*, *Homo sapiens*, *Caenorhabditis elegans*, *Arabidopsis thaliana*, 5 and *Ricinus communis*. The degenerate primers used were as follows using the CODEHOP Blockmaker program (<http://blocks.fhcrc.org/codehop.html>):

a. Protein motif 1: NH<sub>3</sub>- VYDVTEWVKRHPGG -COOH (SEQ ID NO:56)  
 Primer R0 834 (SEQ ID NO:1):  
 10 5'-GTBTAYGAYGTBACCGARTGGGTBAAGCGYCA YCCBGGHGGH-3'  
 B. Protein Motif 2: NH<sub>3</sub>- GASANWWKHQHNVHH -COOH (SEQ ID NO:57)  
 Primer R0835 (Forward) (SEQ ID NO:2):  
 5'-GGHGCYTCCGCYAACTGGTGGAAAGCAYCAGCAYAACGTBCAYCAY-3'  
 Primer R0836 (Reverse) (SEQ ID NO:3):  
 15 5'-RTGRTGVACGTTRTGCTGCTTCCACCAGTTRGCGGARGCDCC-3'  
 C. Protein Motif 3: NH<sub>3</sub>- NYQIEHHLFPTM -COOH (SEQ ID NO:58)  
 Primer R0838 (Reverse) (SEQ ID NO:4)  
 5'-TTGATRGCTARCTYGTGTRGASAARGGVTGGTAC-3'  

20 In addition, two more primers were designed based on the 2nd and 3rd conserved 'Histidine-box' found in known Δ6-desaturases. These were:  
 Primer R0753 (SEQ ID NO:5) 5'-CATCATCATXGGRAAXARRTGRTG-3'  
 Primer R0754 (SEQ ID NO:6) 5'-CTACTACTACTACAYCAYACXTAY ACXAAY-  
 25 3'.  
 The degeneracy code for the oligonucleotide sequences was:  
 B=C,G,T; H=A,C,T; S=C,G; R=A,G; V=A,C,G; Y=C,T; D= A,T,C; X= A,C,G,T

30

Example IIUse of Degenerate Oligonucleotides  
for the Isolation of a Desaturase from a Fungus

To isolate putative desaturase genes, total RNA was isolated using the lithium chloride method (Hoge, et al. 35 (1982) Exp. Mycol. 6:225-232). Approximately 5 μg was reverse transcribed using the SuperScript Preamplification system (LifeTechnologies, Rockville, MD) to produce first strand cDNA. The following primer combinations were used: R0834/836,

RO834/838, RO835/836, RO835/838 and RO753/754 were used in several PCR reactions with different thermocycling parameters and Taq polymerase at annealing temperatures below 60°C, but no bands were produced.

5 In additional attempts to isolate fragments of desaturases, the degenerate primers RO834 /838 (designed with the block maker program) and RO753/754 were used in a 50  $\mu$ l reaction. The following components were combined: 2  $\mu$ l of the first strand cDNA template, 20mM Tris-HCl, pH 8.4, 50mM KCl, 10 1.5mM MgCl<sub>2</sub>, 200  $\mu$ M each deoxyribonucleotide triphosphate, 0.2 pmole final concentration of each primer and cDNA polymerase (Clonetech, Palo Alto, CA). Thermocycling was carried out as follows: an initial denaturation at 94°C for 3 minutes, followed by 35 cycles of; denaturation at 94°C for 30 seconds, 15 annealing at 60°C for 30 seconds and extension at 72°C for 1 minute. This was followed by a final extension at 72°C for 7 minutes. Two faint bands of approximately 1000 bp were detected for primers RO834 /838, while a slightly smaller but more intense band of 800-900 bp was found with the primer pair 20 RO753/754. The reactions were separated on a 1% agarose gel, excised, and purified with the QiaQuick Gel Extraction Kit (Qiagen, Valencia, CA). The staggered ends on these fragments were 'filled-in' using T4 DNA polymerase (LifeTechnologies, Rockville, MD) as per manufacturer's specifications, and these 25 DNA fragments were cloned into the PCR-Blunt vector (Invitrogen, Carlsbad, CA). The recombinant plasmids were transformed into TOP10 supercompetent cells (Invitrogen, Carlsbad, CA) and clones were partially sequenced.

Subsequently, the sequences of clone 30-3 (reaction with 30 RO834 /838) and clone 17-1(reaction with RO753/754) were found to overlap to create a 1313 bp fragment. The fragment was translated and Tfasta used to search the GenBank database. The highest match was *Mortierella alpina*  $\Delta$ 5-desaturase (Genbank accession # AF067654) (27% homology in 202 amino acids), 35 *Spirulina platensis*  $\Delta$ 6-desaturase (Genbank accession number X87094) (30% homology in 121 amino acids), *Dictyostelium discoideum*  $\Delta$ 5-desaturase (Genbank accession number AB02931) (26% homology in 131 amino acids), and *M. alpina*  $\Delta$ 6-desaturase

(accession number AF110510 (30% homology in 86 amino acids). Since there was a reasonable degree of amino acid homology to known desaturases, a full-length gene encoding a potential desaturase was sought to determine its activity when expressed 5 in yeast.

Example III

Isolation of the full length gene sequence from *T. aureum* (ATCC 34304)

10

To find the full-length gene, two separate PCR reactions were carried out in an attempt to determine the two ends of putative desaturase from the cDNA library. For the 3' end of the gene, RO898 (SEQ ID NO:7) (5'-  
15 CCCAGTCACGACGTTGTAAACGACGCCAG-3') (designed based on the sequence of the pBluescript SK(+) vector (Stratagene, La Jolla, CA) was used in a PCR amplification reaction along with a gene specific primer RO930 (SEQ ID NO:8) (5'-  
GACGATTAACAAGGTGATTCCAGGATGTC). In this case, the Advantage 20 -GC cDNA PCR kit (Clonetech, Palo Alto, CA) was used to overcome PCR amplification problems that occur with GC rich sequences (61% for 1313 bp fragment). PCR thermocycling conditions were as follows: the template was initially denatured at 94°C for 3 minutes, followed by 30 cycles of [94°C 25 for 30 seconds, 52°C for 30 seconds, and 72 °C for 1 minute], and finally an extension cycle at 72 °C for 7 minutes with 20 pmoles of each primer. The PCR products thus obtained was resolved on a 1 % agarose gel, excised, and gel purified using the Qiagen Gel Extraction Kit (Qiagen, Valencia, CA). The 30 staggered ends on the fragment was 'filled-in' using T4 DNA polymerase (LifeTechnologies, Rockville, MD) as per manufactures specifications and cloned into the PCR-Blunt vector (Invitrogen, Carlsbad, CA) as described in Example II. Clone 93-3 sequence overlapped the original 1313 bp fragment 35 and was found to contain an open reading frame, a stop codon, and a poly A tail indicating that this was the 3' end of the gene. Two primers were designed based on clone 93-3 sequence near the stop codon with an *Xba*I created site (underlined) as follows: RO973 (SEQ ID NO:9) (5'-

5' GACTAACTCGAGTCACGTGGACCAGGCCGTGAGGTCTT-3') and RO974 (SEQ ID NO:10) (5'- GACTAACTCGAGTTGACGAGGTTGTAT GTTCGGCGGTTGCTT-3'). Two primers were deliberately chosen because RO973, that contained the stop codon, was high in GC 5 (60%) and might not amplify well. On the other hand, RO974, downstream of the stop codon, was much lower in GC (48%).

Following the same protocol as described above to isolate the 5' end of the gene, RO899 (SEQ ID NO:11) (5'- AGCGGATAACAATTTCACACAGGAAACAGC-3') (designed based on the 10 sequence of the pBluescript SK(+) vector) and the gene specific oligonucleotide RO1004 (SEQ ID NO:12) (5'- TGGCTACCGTCGTGCTGGATGCAAGTTCCG-3') were used for amplification of the cDNA library. Amplification was carried out using 10 pmols of each primer and the cDNA polymerase kit (Clonetech, 15 Palo Alto, CA). The reaction conditions included an initial denaturation at 94°C for 1 minute, followed by 30 cycles of [94°C for 30 seconds, 68°C for 3 minutes], and finally an extension cycle at 68°C for 5 minutes. The PCR products thus obtained were cloned into the PCR-Blunt vector (Invitrogen, 20 Carlsbad, CA) following the same protocol as described above. The recombinant plasmids were transformed into TOP10 supercompetent cells (Invitrogen, Carlsbad, CA), and clones were sequenced. Clone 1004-5 contained an open reading frame, several start codons, and overlapped the original 1313 bp 25 sequence indicating that this was the 5' end of the gene.

To isolate the full-length gene, a primer for the 5' end of the putative desaturase was designed with a created EcoRI (underlined) as follows: RO1046 (SEQ ID NO:13) (5'- CGCATGGAATTCATGACGGTCGGTTGACGAAACGGTG-3').

30 To isolate a full-length clone, both RO1046/973 and RO1046/974 were used with cDNA isolated from the library and genomic DNA as a target. Both cDNA polymerase (Clonetech, Palo Alto, CA) and -GC Advantage Polymerase (Clonetech, Palo Alto, CA) were used to amplify their respective targets with 35 10 pmol of primer with the following reaction conditions: an initial denaturation at 94°C for 1 minute, followed by 30 cycles of [94°C for 30 seconds, 68°C for 3 minutes], and finally an extension cycle at 68°C for 5 minutes. The reactions were gel purified, cut with EcoRI/XhoI and cloned

into *EcoRI/XhoI* prepared yeast expression vector pYX242 (Invitrogen, Carlsbad, CA) that had been treated with shrimp alkaline phosphatase (Roche, Indianapolis, IN) to prevent recircularization. Initial analysis of the full-length sequences showed several base changes. Clones 112-3 and 112-5 (designated pRTA7 and 8, respectively) were derived from the amplification with genomic DNA and -GC Advantage polymerase using primers RO1046/974. Clone 110-3 (designated pRTA5) was derived from a reaction with RO1046/973, genomic DNA target and cDNA polymerase. Clone 111-1 (designated pRTA6) was isolated from the reaction using RO1046/974, cDNA target and -GC Advantage polymerase kit. The sequence of these four plasmids, pRTA5 (SEQ ID NO:14), pRTA6 (SEQ ID NO:15), pRTA7 (SEQ ID NO:16), pRTA8 (SEQ ID NO:17) is shown in Figures 3-6, respectively. (Plasmids pRTA7 and pRTA8 were deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110 on April 19, 2001 and were accorded accession numbers PTA-3301 and PTA-3300, respectively.) This putative desaturase of 1548 bp and 515 amino acids (see Figures 7-10 and SEQ ID NOS:18, 19, 20, 21, respectively) had many of the characteristics of described desaturases. The amino acids corresponding to the 5' end of the enzyme are homologous to cytochrome b5. There are also number of histidine boxes at the following amino acids: 178-25 183- (Q)HDGSH (SEQ ID NO:59); 213-219- (Q)HVLGHH (SEQ ID NO:60); 262-265- HPWH (SEQ ID NO:61); 271-275 -HKFQH (SEQ ID NO:62); and 452-457(H)QIEHH (SEQ ID NO:63). At least either an H or a Q precedes three of these histidine boxes which is unusual. *Dictyostelium discoideum* (Genbank accession number AB029311) has two similar boxes [(Q)HVIHHH (SEQ ID NO:64) and (H)QVVHH] (SEQ ID NO:65), while *M. alpina* (Genbank accession number AF067654) has (Q)HMLGHH (SEQ ID NO:66) and *Synechocystis sp.* only has one (H)QVTHH (SEQ ID NO:67). The sequences of the various putative desaturases differed from each other. Several of the base changes resulted in a change in amino acid, as shown in Table 1. These differences could be naturally occurring variants, introduced by PCR mismatch during final amplification, or a PCR error when the

initial cDNA was produced. There are 7 individual amino acid changes between the four plasmids, none of which are shared (see Figure 2A and B, underlined and bold amino acids). These differences could alter the activity of the encoded enzyme.

5

Table 1  
Amino Acid Differences in Different Clones

Amino Acid Number	PRTA5	PRTA6	PRTA7	PRTA8
99	F	S	F	F
280	F	F	L	F
284	F	F	F	S
317	Y	Y	N	Y
332	T	M	M	M
410	T	T	T	A
513	R	W	W	W

10

Example IV  
Expression of Plasmids  
Containing Putative Desaturases in Yeast

All four plasmids were transformed into competent 15 *Saccharomyces cerevisiae* strain 334. Yeast transformation was carried out using the Alkali-Cation Yeast Transformation Kit (BIO 101, Vista, CA) according to conditions specified by the manufacturer. Transformants were selected for leucine auxotrophy on media lacking leucine (DOB [-Leu]). To detect 20 the specific desaturase activity of these clones, transformants were grown in the presence of 50  $\mu$ M specific fatty acid substrates as listed below:

- a. Linoleic acid (18:2n-6) (conversion to alpha-linolenic acid would indicate  $\Delta$ 15-desaturase activity and conversion to gamma-linolenic acid would indicate  $\Delta$ 6-desaturase activity)
- b. Alpha-linolenic acid (18:3n-3) (conversion to stearidonic acid would indicate  $\Delta$ 6-desaturase activity)
- c. Arachidonic acid (20:4n-6) (conversion to eicosapentaenoic acid would indicate  $\Delta$ 17-desaturase activity)
- d. Adrenic acid (22:4n-6) (conversion to  $\omega$ 3-

docosapentaenoic acid would indicate  $\Delta 19$ -activity or conversion to  $\omega 6$ -docosapentaenoic acid would indicate  $\Delta 4$ -desaturase activity.

5. e.  $\omega 3$ -Docosapentaenoic acid (22:5:n-3) (conversion to docosahexaenoic acid would indicate  $\Delta 4$ -desaturase activity.

The negative control strain was *S. cerevisiae* 334 containing the unaltered pYX242 vector, and these were grown simultaneously.

10 The cultures were vigorously agitated (250 rpm) and grown for 48 hours at 24°C in the presence of 50  $\mu$ M (final concentration) of the various substrates in 50 ml of media lacking leucine after inoculation with overnight growth of single colonies in yeast peptone dextrose broth (YPD) at 30°C.

15 The cells were pelleted, and the pellets vortexed in methanol; chloroform was added along with tritridecanoic acid (as an internal standard). These mixtures were incubated for at least an hour at room temperature or at 4°C overnight. The chloroform layer was extracted and filtered through a Whatman filter with 1 gm

20 anhydrous sodium sulfate to remove particulates and residual water. The organic solvents were evaporated at 40°C under a stream of nitrogen. The extracted lipids were then derivitized to fatty acid methyl esters (FAME) for gas chromatography analysis (GC) by adding 2 mls of 0.5 N

25 potassium hydroxide in methanol to a closed tube. The samples were heated to 95°C-100°C for 30 minutes and cooled to room temperature. Approximately 2 ml of 14% boron trifluoride in methanol was added and the heating repeated. After the extracted lipid mixture cooled, 2 ml of water and 1 ml of

30 hexane were added to extract the fatty acid methyl esters (FAME) for analysis by GC. The percent conversion was calculated by dividing the product produced by the sum of (the product produced + the substrate added) and then multiplying by 100.

35 The results showed conversion of  $\omega 3$ -DPA to DHA and ADA to  $\omega 6$ -DPA. This would indicate  $\Delta 4$ -desaturase activity (see Table 2).

**Table 2**  
**Percent Conversion of Different Substrate Concentrations to Product**

5

Clone	25 uM 22:4n-6	50 uM 22:4n-6	100 uM 22:4n-6	25 uM 22:5n-3	50 uM 22:5n-3	100 uM 22:5n-3
PYX242 (control)	0	0	0	0	0	1.28
PRTA5	3.91	0.9	1.24	10.	6.89	3.1
PRTA6	4.69	2.77	1.18	14.26	8.52	4.98
PRTA7	10.97	6.11	3.14	36.34	17.52	9.92
PRTA8	5.55	2.43	0.92	19.44	8.52	4.33

22:4n-6 to 22:5n-6 (Adrenic acid to  $\omega$ 6-Docosapentaenoic acid)  
 22:5n-3 to 22:6n-3 ( $\omega$ 3-Docosapentaenoic acid to Docosahexaenoic acid)

10

In particular, this is the first demonstration a  $\Delta$ 4-desaturase gene with *in vivo* expression data. The conversion for the four clones ranged from 3.91% to 10.97% for production of  $\omega$ 6-DPA from ADA and 10% to 36.34% for production of DHA from  $\omega$ -3DPA. The enzyme appears to be much more active in the production of DHA rather than  $\omega$ 6-DPA, as indicated by the reduced percent conversion, 36.34% vs 10.97 %, respectively, for 25  $\mu$ M of substrate for clone pRTA7. At 100  $\mu$ M concentration of either substrate, the percent conversion (see Table 2) as well as the amount of product produced (data not shown) decreased, indicating that there may be feedback inhibition of the desaturation step by the substrate. The amount of  $\omega$ 3-DPA (22:5n-3) incorporated (as a percent of the total lipid) is similar for all four plasmids (see Table 3, below). However the amount produced as a percent of the total does vary from 2.74 (PRTA5) to 8.11% (PRTA7). The difference in the conversion rates and percent produced could be due to the difference in sequence, hence amino acid variation of the encoded enzyme in the four plasmids.

20

25

Table 3

Fatty Acid as a Percentage of Total Lipid Extracted from Yeast

Clone	22:4(n-6) Incorporated	22:5(n-3) Produced	22:5(n-6) Incorporated	22:6(n-3) Produced
PYX242 (control)	38.96	0	11.2	0
PRTA5	14.5	0.59	19.8	2.74
PRTA6	16.07	0.79	17.97	4.38
PRTA7	39.88	4.91	14.21	8.11
PRTA8	36.94	2.17	17.45	4.25

5

25  $\mu$ M substrate data shown

## Key:

10 22:4(n-6) = Adrenic acid  
 22:5(n-3) =  $\omega$ 3-Docosapentaenoic acid  
 22:5(n-6) =  $\omega$ 6-Docosapentaenoic acid  
 22:6(n-3) = Docosahexaenoic acid

15

This data shows unequivocally that these plasmids indeed encode a  $\Delta$ 4-desaturase, which has preferred activity on conversion of  $\omega$ 3-DPA to DHA activity over conversion of ADA to  $\omega$ 6-DPA.

20

Example V  
Expression of  $\Delta$ 4-desaturase with the  
Mouse Elongase in Yeast

25

The plasmids pRTA7 and pRTA8 (which had the two highest percent conversion) may be individually co-transformed with pRMEL04, a clone that contains a mouse elongase gene from pRAE-84 (see U.S. Patent Application Serial No. 09/624,670 incorporated herein in its entirety by reference). The mouse elongase of 879 base pairs (see Figure 11 (SEQ ID NO:22) and Figure 12 (SEQ ID NO:23)) may be cloned as an EcoRI/SalI fragment in the yeast expression vector pYES2 (Invitrogen, Carlsbad, CA) at the EcoRI/XhoI sites. This elongase of 292 amino acids catalyzes several of the elongation steps in the PUFA pathway, specifically AA to ADA and EPA to  $\omega$ 3-DPA. ADA and  $\omega$ 3-DPA are substrates for the  $\Delta$ 4-desaturase. Yeast transformants may be selected on minimal media lacking leucine

and uracil (DOB[-Leu-Ura]) for selection of  $\Delta 4$ -desaturase (pRTA7 or pRTA8) and pRMEL04 (mouse elongase). Growth and expression of the yeast culture containing pRMEL04 and pRTA7 or pRTA8 in minimal media lacking uracil and leucine and 2% galactose may result in elongation of exogenously added AA to ADA and  $\Delta 4$  desaturation to  $\omega 6$ -DPA. Additionally, supplementation of EPA to the yeast minimal media may result in elongation to  $\omega 3$ -DPA by the elongase which may then be desaturated by the  $\Delta 4$ -desaturase to produce DHA as shown in Figure 1. This has been previously demonstrated with elongases and other desaturases to produce AA and EPA (see PCT application WO 00/12720) and provides parallel experimental data to show that elongation of a substrate and subsequent desaturation can take place in vivo in an organism such as yeast and potentially other organisms. Further, the present data demonstrates the ability of the  $\Delta 4$ -desaturase to work with another enzyme in the PUFA biosynthetic pathway to produce either  $\omega 6$ -DPA or DHA from the precursors AA and EPA, respectively.

20

Example VIHomologue of  $\Delta 4$ -desaturase from *Schizochytrium aggregatum* (ATCC 28209)

25 In parallel to Example II, RNA was isolated by the acid phenol method from *Schizochytrium aggregatum* (*S. aggregatum*) ATCC 28209. Briefly, pellets of *S. aggregatum* were washed with cold deionized water and repelleted for 5 minutes at 3000 rpm. Approximatley 10 ml of TES solution (10 mM Tris-CL pH 7.5, 10 mM EDTA, and 0.5% SDS) was used to resuspend the pellet. Then 10 ml of acid phenol was added and incubation followed for one hour at 65° C. The pellet was placed on ice for 5 minutes, centrifuged for 5 minutes at 1000 x g at 4° C, and the aqueous phase transferred to a new tube. An additional 10 ml of acid phenol was added to the aqueous phase, the mixture vortexed and separated as before. The aqueous phase containing the nucleic acids was transferred to a new tube. Approximately 1ml of sodium acetate pH 5.3 and 25 ml of ice-cold ethanol were added for overnight precipitation at -70 C. The next

day, the tubes were centrifuged for 15 minutes at 14,000 rpm at 4° C and the supernatant decanted. The pellet was washed with 10 ml of 70% ethanol and centrifuged as in the previous step. The pellet was dried and resuspended in 500ul of RNase 5 free deionized water. The RNA was further purified using the Qiagen RNeasy Maxikit (Qiagen, Valencia, CA) as per the manufacturer's protocol.

cDNA was generated using oligo dT with the SuperScript Preamplification system (Life Technologies, Rockville, MD) 10 with 5 ug of RNA from *S. aggregatum*. Since *S. aggregatum* produces large quantities of DHA, a Δ4-desaturase would be required for DHA production. In an identical experiment, primers R0753 (SEQ ID NO:5) and R0754 (SEQ ID NO:6) were used in the same reaction as in Example II to produce a band around 15 800 base pairs. As before the DNA generated from the PCR reaction was separated on a 1% gel, excised, purified, and cloned into the PCR-Blunt vector (Invitrogen, Carlsbad, CA). The DNA sequence generated from clones saa9 and saa5 overlapped to create the sequence saa.con (SEQ ID NO:24 and 20 Figure 13). The translation of the open reading frame of saa.con DNA sequence to an amino acid sequence (SEQ ID NO:25 and Figure 14) aligned with pRTA7 is shown in Figure 15. The amino acid sequence of the Δ4-desaturase from clone pRTA7 has 25 79.1% identity with the translated saa.con sequence over 249 amino acids. This sequence, due to its high identity with a known Δ4-desaturase, is most likely a fragment of a Δ4-desaturase from *S. aggregatum*. This example provides evidence that this procedure can be used to isolate Δ4-desaturases from other organisms.

30

#### Example VII

#### Isolation of Δ4-Desaturase Nucleotide Sequences from *Schizochytrium aggregatum* (ATCC 28209) and *Thraustochytrium aureum* (BICC 7091)

35

To isolate the 5' and 3'-ends, new primers were designed based on the internal sequence of the isolated *S. aggregatum* fragment shown in Example VI. For the 5 prime end of the gene, R01240 (SEQ ID NO:26) (5'-CCC TCG ATG ATG TGG TTG ACG ATG AAC

-3') was used and subsequently 5 prime nested primer RO1239 (SEQ ID NO:27) (5'-CGG AGC ATG GGG TAG GTG CTG AAG AC-3'). For the 3 prime end, RO1236 (SEQ ID NO:28) (5'-CCA ACT GCC GTT ACG CCA GCA AGT -3') was used followed by 3 prime nested primer 5 RO1237 (SEQ ID NO:29) (5'-CAA GCT CTT CTT CAT CGC CCA CTT TTC G-3'), for a second reaction to isolate the other end of the gene. RACE (rapid amplification of cDNA ends) ready cDNA was used as a target for the reactions. To prepare this material, approximately 5 µg of total RNA was used according the 10 manufacturer's direction with the GeneRacer™ kit (Invitrogen, Carlsbad, CA) and Superscript II™ enzyme (Invitrogen, Carlsbad, CA) for reverse transcription to produce cDNA target. For the initial amplification of the ends, the following thermocycling protocol was used in a Perkin Elmer 15 9600: initial melt at 94°C for 2 minutes followed by 5 cycles of 94°C for 30 seconds and 72°C for 3 minutes, 10 cycles of 94°C 30 seconds, 70°C for 30 seconds, and 72°C for 3 minutes and 20 cycles of 94°C for 30 seconds, 68°C for 30 seconds and 72°C for 3 minutes, followed by an extension of 72°C for 10 20 minutes. The first PCR reaction was performed with 10 pMol of RO1240 and 30 pMol GeneRacer™ 5 prime primer (SEQ ID NO:30) (5'- CGA CTG GAG CAC GAG GAC ACT GA-3') or RO1236 and GeneRacer™ 3 prime primer (SEQ ID NO:31) (5'- GCT GTC AAC GAT ACG CTA CGT AAC G-3'). The reaction contained 1 ul of cDNA in 25 a final volume of 50 ul with Platinum Taq™ PCRx (Clonetech, Palo Alto, CA) using MgSO<sub>4</sub> according to the manufacturer's directions. A nested reaction was performed with 1 ul of the initial reaction, 10 pmol of nested primer RO1239 and 30 pmol of the GeneRacer™ nested 5 prime primer (SEQ ID NO:32) (5'- 30 GGA CAC TGA CAT GGA CTG AAG GAG TA-3') or GeneRacer™ nested 3 prime primer (SEQ ID NO:33) (5'- CGC TAC GTA ACG GCA TGA CAG TG -3') and nested primer RO1237 using the same conditions as the first reaction. Agarose gel analysis of the PCR products

showed a band around 800 base pairs for the 5 prime reaction and approximately 600 base pairs for the 3 prime reaction. Subsequent cloning into pCR Blunt (Invitrogen, Carlsbad, CA), transformation into Top10 competent cells (Invitrogen, Carlsbad, CA), and sequencing revealed an open reading frame with both a start and stop codon. Primers RO1241 (SEQ ID NO:34) (5'-GAT ATC GAA TTC ATG ACG GTG GGC GGC GAT GAG G-3') and RO1242 (SEQ ID NO:35) (5'-GTA CTT AAG CTT TCA CTT GGA CTT GGG GTG GTC C-3') with restrictions sites added for cloning (see underlined EcoRI, and HindIII respectively) were used to isolate a full length gene. As shown above, 10 pmol of primers RO1241 and 1242 were used with Platinum Taq<sup>TM</sup> PCRx (Clonetech, PaloAlto, CA) using MgSO<sub>4</sub> according to the manufacturer's protocol with 2  $\mu$ l of the cDNA as target. The thermocycling parameters were as follows: initial melt at 94°C for 2 minutes followed by 5 cycles of 94°C for 30 seconds and 72°C for 2 minutes, 5 cycles of 94°C 30 seconds, 70°C for 2 minutes and 20 cycles of 94°C for 30 seconds, 65°C for 30 seconds and 68°C for 2 minutes, followed by an extension of 68°C for 10 minutes. The large product of the reaction was gel purified using the QiaQuick gel purification kit (Qiagen, Valencia, CA) cut with EcoRI and HindIII and ligated to pYX242 EcoRI/HindIII linearized DNA with the Rapid ligation kit (Roche, Indianapolis, IN) and designated pRSA-1. The clone pRSA1 contained a full length gene of 1530 bp (SEQ ID NO:36, Figure 16) and an open reading frame of 509 amino acids (SEQ ID NO:37, Figure 17). (Plasmid pRSA-1 was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110 on March 27, 2002 and was accorded accession number PTA-4186.)

The second  $\Delta$ 4-desaturase was identified by a partial sequence isolated using the primer combination of RO1201 (SEQ ID NO:38) (5'-CGT GTT CGC TGC CTT TGT CGG AAC TTG CAT CC- 3' and RO1202 (SEQ ID NO:39) (5' - TTG ACA ATA AAC ATG GAG GCG AGG ACC TCT CCG- 3') based on the sequence of pRTA7 (SEQ ID NO:30, Figure 18).

NO:16) as described in Example III. The genomic DNA (gDNA), was prepared as described in Example I, from *Thraustochytrium aureum* (BICC 7091) (Biocon India Ltd., Bangalore, India). PCR amplification was carried out in a 100  $\mu$ l volume containing:

5 5  $\mu$ l of isolated T7091 gDNA, 1.0 U of cDNA Polymerase (Clonetech, PaloAlto, CA) and 10 pMol of primers according the manufacturer's protocol. Thermocycler conditions in Perkin Elmer 9600 were as follows: 94°C for 3 min, then 35 cycles of 94 °C for 30 sec., 60°C for 30 sec., and 72°C for 1 min. PCR

10 was followed by an additional extension at 72°C for 7 minutes. A 600 bp fragment was gel purified, ends filled-in using T4 DNA Polymerase (LifeTechnologies, Rockville, MD, cloned into the pCR-Blunt vector (Invitrogen, Co., Carlsbad, CA), and the recombinant plasmids transformed into TOP10 supercompetent

15 cells (Invitrogen, Carlsbad, CA). Sequencing of the clones revealed high homology with pRTA7 (82.1% in 196 amino acids).

For isolation of a full-length gene, a cDNA library was constructed with mRNA isolated from total RNA using oligo dT cellulose resin. The pBluescript II XR library construction kit (Stratagene, La Jolla, CA) was then used to synthesize double stranded cDNA which was then directionally cloned (5' *NotI*/3' *EcoRI*) into pBluescript II KS(+) vector. The *T. aureum* (BICC 7091) library contained approximately  $1.89 \times 10^8$  clones, each with an average insert size of approximately 1300 bp.

Primers RO1210 (SEQ ID NO:40) (5'-GCT GGT TGG ACT TTG GAC ATG ATT GGA TCC- 3') and RO1211 (SEQ ID NO:41) (5'-TAC ATT GGC AGG CCA ACC ATG TAG AGA ACG - 3') were designed to amplify 5' and 3' sequences, respectively. RO1210/RO899 (SEQ ID NO:11) and RO1211/RO898(SEQ ID NO:7) were set up with cDNA Polymerase (Clonetech, PaloAlto, CA), 5  $\mu$ l of cDNA from the library under the same conditions as described for isolating the original fragment earlier in this example. After cloning and

35 sequencing of fragments an additional internal primer RO1214 (SEQ ID NO:42) (5'-GGA TTC AAT CAT GTC CAA AGT CCA ACC AGC-3')

with RO898 from the vector was used to identify the 5' end of the gene. In a 50  $\mu$ l reaction, 10 pmol of each primer with 5  $\mu$ l of library DNA as target with Platinum Taq<sup>TM</sup> PCRx (Clonetech, PaloAlto, CA) with MgSO<sub>4</sub> was used according to the manufacturer's protocol. The cycling protocol was as follows: an initial melt of 94°C for 5 minutes followed by 35 cycles of 94°C for 45 seconds, 55°C for 30 seconds, 68°C for 2 minutes and an extension cycle of 72°C for 7 minutes.

The full-length  $\Delta$ 4-desaturase from *T. aureum* (BICC 7091) was isolated with 5' primer RO1223 (SEQ ID NO:43) (5'-TCT GAT GAA TTC ATG ACG GCC GGA TTT GAA GAA G-3') and 3' primer RO1224 (SEQ ID NO:44) (5'-GTC TAG CTC GAG TTA GTT CTT GTC CCA GGC AGG CA-3') with added restriction sites EcoRI and XhoI (underlined), respectively, added for cloning purposes. In a 50  $\mu$ l reaction, 10 pmol of each primer, with 5  $\mu$ l of library DNA as target, with Platinum Taq<sup>TM</sup> PCRx (Clonetech, PaloAlto, CA) with MgSO<sub>4</sub> according to the manufacturer's protocol, were used. The cycling protocol was as follows: an initial melt of 94°C for 5 minutes followed by 35 cycles of 94°C for 45 seconds, 55°C for 30 seconds, 68°C for 2 minutes and an extension cycle of 72°C for 7 minutes. The single band was separated on an agarose gel, purified, cut with EcoRI and XhoI, and ligated to pYX242 linearized with the same enzymes. Sequence analysis of the full-length clone designated, pRTA11 (see Figure 18) (SEQ ID NO:45) revealed an open reading frame of 1542 base pairs encoding a protein of 513 amino acids (see Figure 19) (SEQ ID NO:46). (Plasmid pRTA11 was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110 on March 27, 2002 and was accorded accession numbers PTA-4187.)

Example VIII  
Expression of Putative

$\Delta$ 4-Desaturases pRSA1 and pRTA11 in Yeast

Both plasmids were transformed into competent *Saccharomyces cerevisiae* strain 334 and grown as described in Example IV with either 50  $\mu$ M ADA or  $\omega$ 3-DPA. As shown in Table 4, both  $\omega$ 6-DPA and DHA were produced when 334 (pRSA1) or

(pRTA11) was grown with ADA or  $\omega$ 3-DPA, which are the products of a  $\Delta$ 4-desaturation.

5

**Table 4**  
**Fatty Acid as a Percentage of**  
**Total Lipid Extracted from Yeast**

Clone	22:4(n-6) Incorporated	22:5(n-6) Produced	22:5(n-3) Incorporated	22:6(n-3) Produced
PYX242 (control)	15.03	0	20.46	0.25
PYX242 (control)	55.36	0	62.98	0.42
PRTA11	50.73	5.42	42.39	9.17

10 Key:

22:4(n-6) =Adrenic acid  
 22:5(n-3) = $\omega$ 3-Docosapentaenoic acid  
 22:5(n-6) = $\omega$ 6-Docosapentaenoic acid  
 15 22:6(n-3) =Docosahexaenoic acid

When the percent conversion of the substrate to product was calculated as described in Table 5, the preferred substrate, by virtue of the higher percent conversion, was the  $\omega$ 3-DPA to 20 produce DHA. This data shows clearly that these plasmids also encode  $\Delta$ 4-desaturases.

25

**Table 5**  
**Percent Conversion of Two Substrates to Product**

Clone	50 uM 22:4n-6	50 uM 22:5n-3
PYX242 (control)	0	1.2
PRSA1	3.64	9.7
PYX242 (control)	0	0.66
PRTA11	9.65	17.78

22:4n-6 to 22:5n-6 (Adrenic acid to  $\omega$ 6-Docosapentaenoic acid)  
 22:5n-3 to 22:6n-3 ( $\omega$ 3-Docosapentaenoic acid to Docosahexaenoic acid)

30

Example IX

Demonstration of Co-expression of a  $\Delta 4$ -desaturase with a Mouse Elongase in Yeast

As described in Example V, the *T. aureum* (ATCC 34304)  $\Delta 4$ -desaturase was co-transformed with the mouse elongase pRMEL04 (recloned from the plasmid pRAE84 into pYES2).

Table 6 shows that when 10  $\mu$ M of the substrate EPA (20:5n-3) was added, the elongase was able to add two carbons to EPA to produce  $\omega 3$ -DPA, and the desaturase converted  $\omega 3$ -DPA to DHA. No DHA was produced by the control transformation 334(pYX242/pYES2). A small amount of  $\omega 3$ -DPA was seen in the control, but was a contaminant of the added substrate EPA. Thus, *T. aureum*  $\Delta 4$ -desaturase was able to produce a product in a heterologous expression system that was the product of another heterologous enzyme (the mouse elongase) from the PUFA biosynthetic pathway to produce the expected PUFA. This demonstrates that  $\Delta 4$ -desaturase can indeed work with other heterologous enzymes in the PUFA pathway in a heterologous expression system such as yeast.

20

**Table 6**  
**Fatty Acid ( $\mu$ g) Extracted Lipid from Yeast**

Clone	EPA Incorporated	$\omega 3$ -DPA Produced by elongase	DHA Produced by desaturase
PYX242/ pYES2 (control)	59.38	2.54	0
PRTA7/ pRMEL04 (mouse elongase)	47.04	14.76	1.55

25

10  $\mu$ M substrate added

Example IXIsolation of a Novel Desaturase Gene from the Algae *Isochrysis galbana* (CCMP1323)

5        The fatty acid composition of the algae *Isochrysis galbana* (*I. galbana*) (CCMP 1323) was investigated to determine the polyunsaturated fatty acids (PUFAs) produced by this organism. This algae showed a substantial amount of long chain PUFA including omega 3-docosapentaenoic acid (omega 3-DPA, 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). In fact DHA was present in the highest amount representing 19% of the total lipid. Thus, *I. galbana* was predicted to possess a Δ4-desaturase capable of converting omega 3-DPA to DHA. The goal was therefore to isolate the predicted Δ4-desaturase gene from *I. galbana*, and to verify the functionality of the enzyme by expression in an alternate host.

10      Frozen pellets of *I. galbana* were obtained from Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP, West Boothbay Harbor, ME). These pellets were crushed in liquid nitrogen and total RNA was extracted from *I. galbana* by using the Qiagen RNeasy Maxi Kit (Qiagen, as per manufacturers instructions. From this total RNA, mRNA was isolated using oligo dT cellulose resin, which was then used for the construction of a cDNA library using the 15 pBluescript II XR library construction kit (Stratagene, La Jolla, CA). The cDNA thus produced was directionally cloned (5'NotI/3'EcoRI) into pBluescript II KS (+) vector. The *I. galbana* library contained approximately  $9.4 \times 10^4$  clones per μl, each with an average insert size of approximately 1300 bp.

20      Two thousand primary clones from this library were sequenced from the 5' end using the M13 forward primer (SEQ NO ID:47) (5'-AGC GGA TAA CAA TTT CAC ACA GG-3'). Sequencing was carried out using the ABI BigDye sequencing kit (Applied Biosystems, CA) and the MegaBase Capillary DNA sequencer 25 (Amersham biosciences, Piscataway, NJ).

A 647 bp clone containing the 3' end of this novel  $\Delta$ 4-desaturase designated 'iso25-A09' was obtained from sequencing of the 2000 library clones. This fragment shared ~30% amino acid sequence identity with other known delta 5 and delta 6 desaturases. Since this fragment did not contain the stop codon of the gene, additional clones containing the 3' end of this gene were obtained by PCR amplification of the cDNA library (template) using the 3'-end vector primer RO899 (SEQ ID NO:11) and RO1270 (SEQ ID NO:48) (5'- CAC CTG GCT CGA GTC 10 GAC GAT GAT GG -3'). PCR amplification was carried out using Platinum Taq (HF) DNA polymerase (Invitrogen, Carlsbad, CA). Amplification was carried out in a 50  $\mu$ l total volume containing: 1  $\mu$ l of the cDNA library ligation mixture, PCR buffer containing 20 mM Tris-Cl, pH 8.4, 50 mM KCl (final concentration), 200  $\mu$ M each deoxyribonucleotide triphosphate, 10 pmole of each primer, 1.5 mM MgSO<sub>4</sub>, and 0.5  $\mu$ l of Platinum Taq (HF) DNA polymerase. Amplification was carried out as follows using the Perkin Elmer 9600 machine: initial melt at 94°C for 2 minutes followed by 5 cycles of 94°C for 30 seconds and 72°C for 3 minutes, 10 cycles of 94°C 30 seconds, 70°C for 30 seconds, and 72°C for 3 minutes and 20 cycles of 94°C for 30 seconds, 68°C for 30 seconds and 72°C for 3 minutes, followed by an extension of 72°C for 10 minutes. From this amplification no bands were visible which might have been due to the low amounts of this gene in the library. Thus 2  $\mu$ l of this PCR reaction was used as a template for a second PCR reaction involving Platinum Taq (HF) DNA polymerase under that same PCR components as described above. However, this time amplification was carried out as follows: initial denaturation at 94°C for 3 minute, followed by 30 cycles of the following: 94°C for 45 sec, 55°C for 30 sec, 68°C for 2 min. The reaction was terminated at 4°C. A 670 bp PCR band was thus obtained which was gel purified, and cloned into PCR-Blunt vector (Invitrogen, Carlsbad, CA). The recombinant plasmids were

transformed into TOP10 supercompetent cells (Invitrogen, Carlsbad, CA), and clones were sequenced and analyzed. Clones 'iso25-A09-6' and iso25-A09-1' were thus obtained that contained the 3' end of the gene along with the 'TAA' stop 5 codon and the poly-A tail. This clone did overlap with the original 'iso25-A09' fragment.

To isolate the 5' end of this gene, RACE (rapid amplification of cDNA ends) ready cDNA was used as a target for the reactions. To prepare this material, approximately 10  $\mu$ g of total RNA was used according the manufacturer's direction with the GeneRacer™ kit (Invitrogen, Carlsbad, CA) and Superscript II™ enzyme (Invitrogen, Carlsbad, CA) for reverse transcription to produce cDNA target. This cDNA was then use as a template for a PCR reaction involving 30 pmol 15 GeneRacer™ 5' primer (SEQ ID NO:30) (5'- CGA CTG GAG CAC GAG GAC ACT GA-3') in combination with 10 pmols of any one of the following gene-specific primers:

RO1286 (SEQ ID NO:49)  
5'- CGT ACC CGG TGC AAT AGA AGG TGA G -3'  
20 RO1287 (SEQ ID NO:50)  
5'- CCA TCA TCG TCG ACT CGA GCC AGG TG -3'  
RO1288 (SEQ ID NO:51)  
5'- TGT GGA GCC ATG TGG TGC TCG ATC TG -3'  
PCR amplification was carried out using Platinum Taq DNA 25 polymerase (Invitrogen, Carlsbad, CA) in a 50  $\mu$ l total volume containing: 1  $\mu$ l of the RACE-cDNA, PCR buffer containing 20 mM Tris-Cl, pH 8.4, 50 mM KCl (final concentration), 200  $\mu$ M each deoxyribonucleotide triphosphate, 1.5 mM MgSO<sub>4</sub>, and 0.5  $\mu$ l of Platinum Taq DNA polymerase. Amplification was carried out as 30 follows using the Perkin Elmer 9600 machine: initial melt at 94°C for 2 minutes followed by 5 cycles of 94°C for 30 seconds and 72°C for 3 minutes, 10 cycles of 94°C 30 seconds, 70°C for 30 seconds, and 72°C for 3 minutes and 20 cycles of 94°C for 30 seconds, 68°C for 30 seconds and 72°C for 3 minutes, followed

by an extension of 72°C for 10 minutes. All these primer combinations resulted in bands, which were gel purified, filled-in with T4-DNA polymerase, cloned into PCR-blunt vector and transformed into TOP10 supercompetent cells. Sequencing 5 of these clones like 'iso25-A09-33-5', 'iso25-A09-31-3', iso25-A09-30-1' and iso25-A09-32-3' revealed the 5' end of this gene containing the 'ATG' start site, the cytochrome b5 domain and two histidine boxes. These clones overlapped each other and also overlapped the original 'iso25-A09' fragment 10 that contained the third histidine box.

To isolate the full length of this gene both genomic DNA, as well as cDNA obtained (from RACE), were used as templates in PCR reactions with the following primers:

RO 1400 (SEQ ID NO:52)

15 5'- TCA ACA GAA TTC **ATG** TGC AAC GCG GCG CAG GTC GAG ACG CAG - 3'

(This forward primer contained an *EcoRI* site (underlined) along with the 'ATG' start site (bold) suitable for cloning into the yeast expression vector pYX242).

20

RO 1401 (SEQ ID NO:53)

5'- AAA AGA AAG CTT **TTA** GTC CGC CTT GAC CGT GTC GAC CAA AGC - 3'

(This reverse primer contained a *HindIII* site (underlined) 25 along with the 'TAA' stop site (bold) for cloning into pYX242). PCR amplification was carried out using Advantage-GC cDNA polymerase (Clonetech, Palo Alto, CA) in a 50 µl total volume containing: 1 µl of the RACE-cDNA or 2 µl of genomic DNA, PCR buffer containing [40 mM Tricine-KOH pH 9.2, 15 mM KOAc (final concentration), 3.5 mM Mg(OAc)<sub>2</sub>, 5% DMSO, 3.75 µg/ml BSA, 200 µM each deoxyribonucleotide triphosphate, 1M GC-melt, and 1 µl of Advantage-GC cDNA polymerase. The thermocycling protocol included an initial denaturation at 94°C for 1 min, followed by 30 cycles of the following

[denaturation at 94°C for 30 seconds and annealing at 68°C for 3 minutes], a final extension at 68°C for 5 minutes, followed by termination at 4°C.

5        A ~1.35 kb band was obtained which was gel purified, digested with the restriction enzymes *EcoRI/HindIII* for 2 hours, cleaned through the QiaQuick PCR purification kit (Qiagen, Valencia, CA), and cloned into the pYX242 yeast expression vector (Novagen, Madison, WI) previously digested 10 with *EcoRI/HindIII*. This construct was labeled pRIG6 and consisted of the 'iso25-A09' full length gene isolated from RACE-derived cDNA and the pYX242 vector. This was transformed into yeast SC334 for expression studies.

15      The full length gene of 'iso25-A09' present in pRIG6 was 1302 bp in length (SEQ ID NO:54) (Figure 20) and encoded a protein of 433 amino acids (SEQ ID NO:55) (Figure 21). A tFastA search of the deduced protein sequence of this gene showed the protein to have 30.6% identity with the Δ5-desaturase from *I. galbana* (U.S. Patent Appln. No. 10/054,534 20 incorporated in its entirety by reference). Also the predicted protein of this gene was 30.8% identical to the Δ4-desaturase from *Thraustochytrium aureum* (ATCC 34304) (Figure 22). (Further, the DNA sequence of the gene was found to exhibit 42.37% sequence identity to the nucleotide sequence 25 encoding the *T. aureum* (ATCC 34304) Δ4-desaturase, 43% identity to the nucleotide sequence encoding the *S. aggregatum* (ATCC 28209) Δ4-desaturase, and 39.7% identity to the nucleotide sequence encoding the *T. aureum* (BICC7091) Δ4-desaturase sequence.) Like all front-end desaturating enzyme 30 genes like Δ5- and Δ6-desaturase, this gene contains a cytochrome b5 domain within the 5'-end of its sequence. This cytochrome b5 is thought to function as the immediate electron donor for the desaturases, and functions in a number of oxidation-reduction reactions involving NADH-dependent 35 desaturation. This gene also possessed the three histidine-

rich motifs that are present in all membrane-bound desaturases. These are present at position 153 to 158 (HMGHH) (SEQ ID NO:71), 188 to 193 (HNKHH) (SEQ ID NO:72), and 347 to 352 (QIEHH) (SEQ ID NO:73). These histidine-rich boxes are believed to co-ordinate the diiron-oxo structure at the enzyme's active site, and are necessary for enzyme activity. These features are consistent with this gene product being a member of the membrane-bound desaturase/hydroxylase family of the diiron-oxo proteins (3) and also being a front-end desaturating enzyme. The G+C content of this gene is 64.2%.

Example X

Expression of pRIG6, a Novel Desaturase from *Isochrysis galbana* (CCMP 1323); in Yeast

To determine the substrate specificity and the class of reaction catalyzed by a novel desaturase from *I. galbana*, the pRIG6 construct was heterologously expressed in a *Saccharomyces cerevisiae* (SC334), as described below. Since *S. cerevisiae* cannot synthesize fatty acids beyond oleic acid (OA, 18:1 n-9), it is an ideal system to use to determine enzyme activity on substrates longer than OA since no background enzyme activity will be detected. Here, substrates can be exogenously supplied to the host, taken up by the cell and acted on by the expressed protein of the transformed gene.

Clone pRIG6, which consisted of the full-length 'iso25-A09' desaturase from *I. galbana* cloned into pYX242, was transformed into *Saccharomyces cerevisiae* (SC334) using the Alkali-Cation Yeast Transformation kit (BIO 101, Vista, CA). (Plasmid pRIG6 was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110 on April 5, 2002 and was accorded accession number PTA-4209.) Transformants were selected for leucine auxotrophy on media lacking leucine (DOB [-Leu]). To detect the specific desaturase activity of these clones, transformants were grown

in the presence of 50  $\mu$ M specific fatty acid substrates as listed below:

- a. Linoleic acid (LA, 18:2n-6) - conversion to  $\alpha$ -linolenic acid (ALA, 18:3n-3) indicates  $\Delta$ 15- desaturase activity; conversion to gamma-linolenic acid indicates  $\Delta$ 6- desaturase activity.
- b. Dihomo-gamma-linolenic acid (20:3n-6)- conversion to eicosatetraenoic acid (ETA, 20:4n-3) indicates  $\Delta$ 17- desaturase activity; conversion to arachidonic acid (ARA, 20:4n-6) indicates  $\Delta$ 5-desaturase activity.
- c. Omega-6-eicosadienoic acid (20:2n-6)-conversion to Dihomo-gamma-linolenic acid (20:3n-6) indicates  $\Delta$ 8- desaturase activity.
- d. Adrenic acid (22:4n-6)-conversion to  $\omega$ 6-docosapentaenoic acid (22:5n-6) indicates  $\Delta$ 4- desaturase activity.
- e. Omega 3-docosapentaenoic acid (22:5n-3)-conversion to Docosahexaenoic acid (22:6n-3) indicates  $\Delta$ 4-desaturase activity.

20

The negative control strain consisted of *S. cerevisiae* transformed with the pYX242 vector, and these cultures were grown simultaneously and analyzed.

The cultures were vigorously agitated (250 rpm) and grown 25 for 48 hours at 24°C in the presence of 50  $\mu$ M (final concentration) of the various substrates (Table 7). The cells were spun down, washed once in distilled water, and the pellets vortexed in methanol; chloroform was added along with tridecanoin (as an internal standard). These mixtures were 30 incubated for at least an hour at room temperature, or at 4°C overnight. The chloroform layer was extracted and filtered through a Whatman filter with 1 gm anhydrous sodium sulfate to remove particulates and residual water. The organic solvents were evaporated at 40°C under a stream of nitrogen. The 35 extracted lipids were then derivitized to fatty acid methyl

esters (FAME) for gas chromatography analysis (GC) by adding 2 ml 0.5 N potassium hydroxide in methanol to a closed tube. The samples were heated to 95°C-100°C for 30 minutes and cooled to room temperature. Approximately 2 ml 14% borontrifluoride in methanol was added and the heating repeated. After the extracted lipid mixture cooled, 2 ml of water and 1 ml of hexane were added to extract the FAME for analysis by GC. The percent conversion was calculated using the formula:

$$10 \quad \% \text{ Conversion} = \frac{[\% \text{ Product}]}{[\% \text{ Product} + \% \text{ Substrate}]} \times 100$$

Table 7 shows the substrate specificity of the novel desaturase expressed in yeast. Here, the expressed pRIG6 clone was capable of converting 15.3% of  $\omega 3$ -docosapentaenoic acid (22:5n-3) to docosahexaenoic acid (22:5 n-3), indicating that the gene was a  $\Delta 4$ -desaturase. In addition, this enzyme was capable of converting 11% of adrenic acid (22:4n-6) to  $\omega 6$ -docosapentaenoic acid (22:5n-6), which also indicated  $\Delta 4$ -desaturase activity.

The fatty acids of interest are represented as a percentage of the total lipids extracted from yeast. GC/MS was employed to identify the products. Under these conditions, the clones did not exhibit other desaturase activities. This confirmed the gene isolated to be a novel  $\Delta 4$ -desaturase gene. No background substrate conversion was detected with using just the vector alone. This data indicates that this novel  $\Delta 4$ -desaturase can be expressed in a heterologous system and would thus be useful in the production of transgenic oil containing DHA.

Table 7

Isochrysis galbana (CCMP 1323) Delta 4-Desaturase Expression  
in Baker's Yeast at 24°C

Clone	Desaturase activity	Substrate* Incorpor.	Substrate Produced	% Conversion of Substrate
PRIG6 (pYX242 + Delta 4	Δ6	LA (8.35%)	GLA (0%)	0
	Δ5	DGLA (16.34%)	AA (0.29%)	0
	Δ8	ω6-EDA (19.53%)	DGLA (0%)	0
	Δ4	ADA (23.93%)	ω6-DPA (3.15%)	11%
	Δ4	ω3-DPA (32.57%)	DHA (5.89%)	15.3%
Control (pYX242)	Δ6	LA (9.18%)	GLA (0%)	0
	Δ5	DGLA (10.5%)	AA (0%)	0
	Δ8	ω6-EDA (16.56%)	DGLA (0%)	0
	Δ4	ADA (15.55%)	ω6-DPA (0%)	0
	Δ4	ω3-DPA (26.03%)	DHA (0.29%)	0

5 \* 50 μM substrate used

Numbers in parenthesis represent fatty acid as a percentage of total lipids from yeast

Key:

10 LA= Linoleic acid (18:2n-6)  
 GLA= Gamma-linolenic acid (18:3n-6)  
 DGLA= Dihomo-gamma-linolenic acid (20:3n-6)  
 AA= Arachidonic acid (20:4n-6)  
 ω6-EDA= omega-6 Eicosadienoic acid (20:2n-6)  
 15 ADA= Adrenic acid (22:4n-6)  
 ω3-DPA= omega-3 Docosapentaenoic acid (22:5n-6)

$\omega$ 6-DPA= omega-6 Docosapentaenoic acid (22:5n-3)

DHA= Docosahexaenoic acid (22:6n-3)

#### Nutritional Compositions

5 The PUFAs described in the Detailed Description may be utilized in various nutritional supplements, infant formulations, nutritional substitutes and other nutritional solutions.

10 I. INFANT FORMULATIONS

A. Isomil® Soy Formula with Iron:

Usage: As a beverage for infants, children and adults with an allergy or sensitivity to cows milk. A feeding for patients 15 with disorders for which lactose should be avoided: lactase deficiency, lactose intolerance and galactosemia.

Features:

- Soy protein isolate to avoid symptoms of cow's-milk-protein allergy or sensitivity.
- Lactose-free formulation to avoid lactose-associated diarrhea.
- Low osmolality (240 mOs/kg water) to reduce risk of osmotic diarrhea.
- Dual carbohydrates (corn syrup and sucrose) designed to enhance carbohydrate absorption and reduce the risk of exceeding the absorptive capacity of the damaged gut.
- 1.8 mg of Iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.
- Recommended levels of vitamins and minerals.
- Vegetable oils to provide recommended levels of essential fatty acids.
- Milk-white color, milk-like consistency and pleasant aroma.

Ingredients: (Pareve) 85% water, 4.9% corn syrup, 2.6% sugar (sucrose), 2.1 % soy oil, 1.9% soy protein isolate, 1.4% coconut oil, 0.15% calcium citrate, 0. 11 % calcium phosphate tribasic, potassium citrate, potassium phosphate monobasic, potassium chloride, mono- and disglycerides, soy lecithin, carrageenan, ascorbic acid, L-methionine, magnesium chloride, potassium phosphate dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

B. Isomil® DF Soy Formula For Diarrhea:

Usage: As a short-term feeding for the dietary management of diarrhea in infants and toddlers.

Features:

- First infant formula to contain added dietary fiber from soy fiber specifically for diarrhea management.
- Clinically shown to reduce the duration of loose, watery stools during mild to severe diarrhea in infants.
- Nutritionally complete to meet the nutritional needs of the infant.
- Soy protein isolate with added L-methionine meets or exceeds an infant's requirement for all essential amino acids.
- Lactose-free formulation to avoid lactose-associated diarrhea.
- Low osmolality (240 mOsm/kg water) to reduce the risk of osmotic diarrhea.

- Dual carbohydrates (corn syrup and sucrose) designed to enhance carbohydrate absorption and reduce the risk of exceeding the absorptive capacity of the damaged gut.
- 5 -Meets or exceeds the vitamin and mineral levels recommended by the Committee on Nutrition of the American Academy of Pediatrics and required by the Infant Formula Act.
- 1.8 mg of iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.
- 10 -Vegetable oils to provide recommended levels of essential fatty acids.

15 Ingredients: (Pareve) 86% water, 4.8% corn syrup, 2.5% sugar (sucrose), 2.1% soy oil, 2.0% soy protein isolate, 1.4% coconut oil, 0.77% soy fiber, 0.12% calcium citrate, 0.11% calcium phosphate tribasic, 0.10% potassium citrate, potassium chloride, potassium phosphate monobasic, mono and diglycerides, soy lecithin, 20 carrageenan, magnesium chloride, ascorbic acid, L-methionine, potassium phosphate dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, 25 vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

30 C. Isomil® SF Sucrose-Free Soy Formula With Iron:

- Usage: As a beverage for infants, children and adults with an allergy or sensitivity to cow's-milk protein or an intolerance to sucrose. A feeding for patients with disorders for which lactose and sucrose should be avoided.

Features:

- Soy protein isolate to avoid symptoms of cow's-milk-protein allergy or sensitivity.
- 5 -Lactose-free formulation to avoid lactose-associated diarrhea (carbohydrate source is Polycose® Glucose Polymers).
- Sucrose free for the patient who cannot tolerate sucrose.
- 10 -Low osmolality (180 mOsm/kg water) to reduce risk of osmotic diarrhea.
- 1.8 mg of iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.
- Recommended levels of vitamins and minerals.
- 15 -Vegetable oils to provide recommended levels of essential fatty acids.
- Milk-white color, milk-like consistency and pleasant aroma.

20 Ingredients: (Pareve) 75% water, 11.8% hydrolyzed cornstarch, 4.1% soy oil, 4.1 % soy protein isolate, 2.8% coconut oil, 1.0% modified cornstarch, 0.38% calcium phosphate tribasic, 0.17% potassium citrate, 0.13% potassium chloride, mono- and diglycerides, soy lecithin, 25 magnesium chloride, ascorbic acid, L-methionine, calcium carbonate, sodium chloride, choline chloride, carrageenan, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, 30 vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

35 D. Isomil® 20 Soy Formula With Iron Ready To Feed,

20 Cal/fl oz.:

Usage: When a soy feeding is desired.

5                   Ingredients: (Pareve) 85% water, 4.9% corn syrup, 2.6% sugar(sucrose), 2.1 % soy oil, 1.9% soy protein isolate, 1.4% coconut oil, 0.15% calcium citrate, 0. 11% calcium phosphate tribasic, potassium citrate, potassium phosphate monobasic, potassium chloride, mono- and diglycerides, soy lecithin, carrageenan, ascorbic acid, 10 L-methionine, magnesium chloride, potassium phosphate dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, 15 thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

20                   E.    Similac® Infant Formula:

Usage: When an infant formula is needed: if the decision is made to discontinue breastfeeding before age 1 year, if a supplement to breastfeeding is needed or as a 25 routine feeding if breastfeeding is not adopted.

Features:

30                   -Protein of appropriate quality and quantity for good growth; heat-denatured, which reduces the risk of milk-associated enteric blood loss.

-Fat from a blend of vegetable oils (doubly homogenized), providing essential linoleic acid that is easily absorbed.

35                   -Carbohydrate as lactose in proportion similar to that of human milk.

-Low renal solute load to minimize stress on developing organs.

-Powder, Concentrated Liquid and Ready To Feed forms.

5        Ingredients: (-D) Water, nonfat milk, lactose, soy oil, coconut oil, mono- and diglycerides, soy lecithin, ascorbic acid, carrageenan, choline chloride, taurine, m-inositol, alpha-tocopheryl acetate, zinc sulfate, niacinamide, ferrous sulfate, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, 10 folic acid, manganese sulfate, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

15        F.     Similac® NeoCare Premature Infant Formula With Iron:

Usage: For premature infants' special nutritional needs after hospital discharge. Similac NeoCare is a nutritionally complete formula developed to 20 provide premature infants with extra calories, protein, vitamins and minerals needed to promote catch-up growth and support development.

Features:

25        -Reduces the need for caloric and vitamin supplementation. More calories (22 Cal/fl oz) than standard term formulas (20 Cal/fl oz).  
-Highly absorbed fat blend, with medium-chain triglycerides  
30        (MCToil) to help meet the special digestive needs of premature infants.  
-Higher levels of protein, vitamins and minerals per 100 calories to extend the nutritional support initiated in-hospital.

-More calcium and phosphorus for improved bone mineralization.

5                   Ingredients: -D Corn syrup solids, nonfat milk, lactose, whey protein concentrate, soy oil, high-oleic safflower oil, fractionated coconut oil (medium chain triglycerides), coconut oil, potassium citrate, calcium phosphate tribasic, calcium carbonate, ascorbic acid, magnesium chloride, potassium chloride, sodium chloride, 10 taurine, ferrous sulfate, m-inositol, choline chloride, ascorbyl palmitate, L-carnitine, alpha-tocopheryl acetate, zinc sulfate, niacinamide, mixed tocopherols, sodium citrate, calcium pantothenate, cupric sulfate, thiamine chloride hydrochloride, vitamin A palmitate, 15 beta carotene, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

20                   G.    Similac Natural Care Low-Iron Human Milk Fortifier Ready To Use, 24 Cal/fl oz.:

25                   Usage: Designed to be mixed with human milk or to be fed alternatively with human milk to low-birth-weight infants.

30                   Ingredients: -D Water, nonfat milk, hydrolyzed cornstarch, lactose, fractionated coconut oil (medium-chain triglycerides), whey protein concentrate, soy oil, coconut oil, calcium phosphate tribasic, potassium citrate, magnesium chloride, sodium citrate, ascorbic acid, calcium carbonate, mono and diglycerides, soy lecithin, carrageenan, choline chloride, m-inositol, taurine, niacinamide, L-carnitine, alpha tocopheryl acetate, zinc sulfate, potassium chloride, calcium pantothenate, ferrous sulfate, cupric sulfate, 35

riboflavin, vitamin A palmitate, thiamine chloride hydrochloride, pyridoxine hydrochloride, biotin, folic acid, manganese sulfate, phylloquinone, vitamin D3, sodium selenite and cyanocobalamin.

5 Various PUFAs of this invention can be substituted and/or added to the infant formulae described above and to other infant formulae known to those in the art.

## II. NUTRITIONAL FORMULATIONS

10

### A. ENSURE®

Usage: ENSURE is a low-residue liquid food designed primarily as an oral nutritional supplement to be used with or between meals or, in appropriate amounts, as a meal replacement. ENSURE is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets. Although it is primarily an oral supplement, it can be fed by tube.

20 Patient Conditions:

- For patients on modified diets
- For elderly patients at nutrition risk
- For patients with involuntary weight loss
- For patients recovering from illness or surgery
- For patients who need a low-residue diet

30 Ingredients: -D Water, Sugar (Sucrose), Maltodextrin (Corn), Calcium and Sodium Caseinates, High-Oleic Safflower Oil, Soy Protein Isolate, Soy Oil, Canola Oil, Potassium Citrate, Calcium Phosphate Tribasic, Sodium Citrate, Magnesium Chloride, Magnesium Phosphate Dibasic, Artificial Flavor, Sodium Chloride, Soy Lecithin, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Gellan Gum, Niacinamide,

35

5                   Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate,  
Vitamin A Palmitate, Thiamine Chloride Hydrochloride,  
Pyridoxine Hydrochloride, Riboflavin, Folic Acid, Sodium  
Molybdate, Chromium Chloride, Biotin, Potassium Iodide,  
Sodium Selenate.

B. ENSURE® BARS:

10                  Usage: ENSURE BARS are complete, balanced nutrition for  
supplemental use between or with meals. They provide a  
delicious, nutrient-rich alternative to other snacks.

15                  ENSURE BARS contain <1 g lactose/bar, and Chocolate Fudge  
Brownie flavor is gluten-free. (Honey Graham Crunch  
flavor contains gluten.)

15                  Patient Conditions:

20                  -For patients who need extra calories, protein, vitamins  
and minerals.  
-Especially useful for people who do not take in enough  
calories and nutrients.  
-For people who have the ability to chew and swallow  
-Not to be used by anyone with a peanut allergy or any  
type of allergy to nuts.

25                  Ingredients: Honey Graham Crunch -- High-Fructose Corn  
Syrup, Soy Protein Isolate, Brown Sugar, Honey,  
Maltodextrin (Corn), Crisp Rice (Milled Rice,  
Sugar [Sucrose], Salt [Sodium Chloride] and Malt), Oat  
Bran, Partially Hydrogenated Cottonseed and Soy Oils, Soy  
Polysaccharide, Glycerine, Whey Protein Concentrate,  
30                  Polydextrose, Fructose, Calcium Caseinate, Cocoa Powder,  
Artificial Flavors, Canola Oil, High-Oleic Safflower Oil,  
Nonfat Dry Milk, Whey Powder, Soy Lecithin and Corn Oil.  
Manufactured in a facility that processes nuts.

Vitamins and Minerals: Calcium Phosphate Tribasic, Potassium Phosphate Dibasic, Magnesium Oxide, Salt (Sodium Chloride), Potassium Chloride, Ascorbic Acid, Ferric Orthophosphate, Alpha-Tocopheryl Acetate, 5 Niacinamide, Zinc Oxide, Calcium Pantothenate, Copper Gluconate, Manganese Sulfate, Riboflavin, Beta Carotene, Pyridoxine Hydrochloride, Thiamine Mononitrate, Folic Acid, Biotin, Chromium Chloride, Potassium Iodide, Sodium Selenate, 10 Sodium Molybdate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

Protein: Honey Graham Crunch - The protein source is a blend of soy protein isolate and milk proteins.

15	Soy protein isolate	74%
	Milk proteins	26%

Fat: Honey Graham Crunch - The fat source is a blend of 20 partially hydrogenated cottonseed and soybean, canola, high oleic safflower, oils, and soy lecithin.

	Partially hydrogenated cottonseed and soybean oil	76%
25	Canola oil	8%
	High-oleic safflower oil	8%
	Corn oil	4%
	Soy lecithin	4%
30	Carbohydrate: Honey Graham Crunch - The carbohydrate source is a combination of high-fructose corn syrup, brown sugar, maltodextrin, honey, crisp rice, glycerine, soy polysaccharide, and oat bran.	

35	High-fructose corn syrup	24%
	Brown sugar	21%
	Maltodextrin	12%

	Honey	11%
	Crisp rice	9%
	Glycerine	9%
	Soy Polysaccharide	7%
5	Oat bran	7%

C. ENSURE® HIGH PROTEIN:

Usage: ENSURE HIGH PROTEIN is a concentrated, high-protein liquid food designed for people who require additional calories, protein, vitamins, and minerals in their diets. It can be used as an oral nutritional supplement with or between meals or, in appropriate amounts, as a meal replacement. ENSURE HIGH PROTEIN is lactose- and gluten-free, and is suitable for use by people recovering from general surgery or hip fractures and by patients at risk for pressure ulcers.

Patient Conditions:

-For patients who require additional calories, protein, vitamins, and minerals, such as patients recovering from general surgery or hip fractures, patients at risk for pressure ulcers, and patients on low-cholesterol diets.

Features:

- Low in saturated fat
- Contains 6 g of total fat and < 5 mg of cholesterol per serving
- Rich, creamy taste
- Excellent source of protein, calcium, and other essential vitamins and minerals
- For low-cholesterol diets
- Lactose-free, easily digested

Ingredients:

Vanilla Supreme: -D Water, Sugar (Sucrose), Maltodextrin (Corn), Calcium and Sodium Caseinates, High-Oleic Safflower Oil, Soy Protein Isolate, Soy Oil, Canola Oil, 5 Potassium Citrate, Calcium Phosphate Tribasic, Sodium Citrate, Magnesium Chloride, Magnesium Phosphate Dibasic, Artificial Flavor, Sodium Chloride, Soy Lecithin, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Gellan Gum, 10 Niacinamide, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Folic Acid, Sodium Molybdate, Chromium Chloride, Biotin, Potassium Iodide, 15 Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

Protein:

The protein source is a blend of two high-biologic-value 20 proteins: casein and soy.

Sodium and calcium caseinates	85%
Soy protein isolate	15%

Fat:

The fat source is a blend of three oils: high-oleic 25 safflower, canola, and soy.

High-oleic safflower oil	40%
Canola oil	30%
Soy oil	30%

The level of fat in ENSURE HIGH PROTEIN meets American 35 Heart Association (AHA) guidelines. The 6 grams of fat in ENSURE HIGH PROTEIN represent 24% of the total calories, with 2.6% of the fat being from saturated fatty acids and

7.9% from polyunsaturated fatty acids. These values are within the AHA guidelines of < 30% of total calories from fat, < 10% of the calories from saturated fatty acids, and < 10% of total calories from polyunsaturated fatty acids.

5.

#### Carbohydrate:

10 ENSURE HIGH PROTEIN contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla supreme, chocolate royal, wild berry, and banana), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance. Vanilla and other nonchocolate flavors are available.

15

Sucrose	60%
Maltodextrin	40%

#### Chocolate:

20

Sucrose	70%
Maltodextrin	30%

#### D. ENSURE® LIGHT

25 Usage: ENSURE LIGHT is a low-fat liquid food designed for use as an oral nutritional supplement with or between meals. ENSURE LIGHT is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.

30

#### Patient Conditions:

35 -For normal-weight or overweight patients who need extra nutrition in a supplement that contains 50% less fat and 20% fewer calories than ENSURE.

-For healthy adults who do not eat right and need extra nutrition.

Features:

- 5        -Low in fat and saturated fat
- Contains 3 g of total fat per serving and < 5 mg cholesterol
- Rich, creamy taste
- Excellent source of calcium and other essential vitamins and minerals
- 10       -For low-cholesterol diets
- Lactose-free, easily digested

Ingredients:

15       French Vanilla: -D Water, Maltodextrin (Corn), Sugar (Sucrose), Calcium Caseinate, High-Oleic Safflower Oil, Canola Oil, Magnesium Chloride, Sodium Citrate, Potassium Citrate, Potassium Phosphate Dibasic, Magnesium Phosphate Dibasic, Natural and Artificial Flavor, Calcium Phosphate Tribasic, Cellulose Gel, Choline Chloride, Soy Lecithin, Carrageenan, Salt (Sodium Chloride), Ascorbic Acid, Cellulose Gum, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Zinc Sulfate, Niacinamide, Manganese Sulfate, Calcium Pantothenate, Cupric Sulfate, Thiamine Chloride Hydrochloride, Vitamin A Palmitate, Pyridoxine Hydrochloride, Riboflavin, Chromium Chloride, Folic Acid, Sodium Molybdate, Biotin, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

20

25       Protein:

The protein source is calcium caseinate.

30       Calcium caseinate

100%

35

## Fat:

The fat source is a blend of two oils: high-oleic safflower and canola.

5

High-oleic safflower oil	70%
Canola oil	30%

10 The level of fat in ENSURE LIGHT meets American Heart Association (AHA) guidelines. The 3 grams of fat in ENSURE LIGHT represent 13.5% of the total calories, with 1.4% of the fat being from saturated fatty acids and 2.6% from polyunsaturated fatty acids. These values are within the AHA guidelines of < 30% of total calories from 15 fat, < 10% of the, calories from saturated fatty acids, and < 10% of total calories from polyunsaturated fatty acids.

20

## Carbohydrate:

ENSURE LIGHT contains a combination of maltodextrin and sucrose. The chocolate flavor contains corn syrup as well. The mild sweetness and flavor variety (French vanilla, chocolate supreme, strawberry swirl), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, 25 and orange, help to prevent flavor fatigue and aid in patient compliance.

30

Vanilla and other nonchocolate flavors:

Sucrose	51%
Maltodextrin	49%

## Chocolate:

	Sucrose	47.0%
	Corn Syrup	26.5%
5	Maltodextrin	26.5%

## Vitamins and Minerals:

An 8-fl-oz serving of ENSURE LIGHT provides at least 25% of the RDIs for 24 key vitamins and minerals.

10

## Caffeine:

Chocolate flavor contains 2.1 mg caffeine/8 fl oz.

## E. ENSURE PLUS®

15 Usage: ENSURE PLUS is a high-calorie, low-residue liquid food for use when extra calories and nutrients, but a normal concentration of protein, are needed. It is designed primarily as an oral nutritional supplement to be used  
20 with or between meals or, in appropriate amounts, as a meal replacement. ENSURE PLUS is lactose- and gluten-free. Although it is primarily an oral nutritional supplement, it can be fed by tube.

25

## Patient Conditions:

-For patients who require extra calories and nutrients, but a normal concentration of protein, in a limited volume.  
30 -For patients who need to gain or maintain healthy weight.

## Features:

-Rich, creamy taste  
-Good source of essential vitamins and minerals

35

## Ingredients:

Vanilla: -D Water, Corn Syrup, Maltodextrin (Corn), Corn Oil, Sodium and Calcium Caseinates, Sugar (Sucrose), Soy Protein Isolate, Magnesium Chloride, Potassium Citrate, Calcium Phosphate Tribasic, Soy Lecithin, Natural and Artificial Flavor, Sodium Citrate, Potassium Chloride, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, 5 Niacinamide, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Chromium Chloride, Sodium Molybdate, 10 Potassium Iodide, Sodium Selenite, Phylloquinone, 15 Cyanocobalamin and Vitamin D3.

## Protein:

The protein source is a blend of two high-biologic-value proteins: casein and soy. 20

Sodium and calcium caseinates	84%
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Soy protein isolate	16%
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## Fat:

25 The fat source is corn oil.

Corn oil	100%
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## Carbohydrate:

30 ENSURE PLUS contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla, chocolate, strawberry, coffee, buffer pecan, and eggnog), plus VARI-

FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

5       Vanilla, strawberry, butter pecan, and coffee flavors:

Corn Syrup	39%
Maltodextrin	38%
Sucrose	23%

10

Chocolate and eggnog flavors:

Corn Syrup	36%
Maltodextrin	34%
Sucrose	30%

Vitamins and Minerals:

An 8-fl-oz serving of ENSURE PLUS provides at least 15% of the RDIs for 25 key Vitamins and minerals.

20       Caffeine:

Chocolate flavor contains 3.1 mg Caffeine/8 fl oz. Coffee flavor contains a trace amount of caffeine.

F. ENSURE PLUS® HN

25

Usage: ENSURE PLUS HN is a nutritionally complete high-calorie, high-nitrogen liquid food designed for people with higher calorie and protein needs or limited volume tolerance. It may be used for oral supplementation or for total nutritional support by tube. ENSURE PLUS HN is lactose- and gluten-free.

30

Patient Conditions:

35       -For patients with increased calorie and protein needs, such as following surgery or injury.

-For patients with limited volume tolerance and early satiety.

Features:

5       -For supplemental or total nutrition  
-For oral or tube feeding  
-1.5 CaV/mL,  
-High nitrogen  
-Calorically dense

10       Ingredients:

Vanilla: -D Water, Maltodextrin (Corn), Sodium and  
Calcium Caseinates, Corn Oil, Sugar (Sucrose), Soy  
Protein Isolate, Magnesium Chloride, Potassium Citrate,  
15       Calcium Phosphate Tribasic, Soy Lecithin, Natural and  
Artificial Flavor, Sodium Citrate, Choline Chloride,  
Ascorbic Acid, Taurine, L-Carnitine, Zinc Sulfate,  
Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide,  
Carrageenan, Calcium Pantothenate, Manganese Sulfate,  
20       Cupric Sulfate, Thiamine Chloride Hydrochloride,  
Pyridoxine Hydrochloride, Riboflavin, Vitamin A  
Palmitate, Folic Acid, Biotin, Chromium Chloride, Sodium  
Molybdate, Potassium Iodide, Sodium Selenite,  
Phylloquinone, Cyanocobalamin and Vitamin D3.

25       G. ENSURE® POWDER:

Usage: ENSURE POWDER (reconstituted with water) is a  
low-residue liquid food designed primarily as an oral  
30       nutritional supplement to be used with or between  
meals. ENSURE POWDER is lactose- and gluten-free, and  
is suitable for use in modified diets, including low-  
cholesterol diets.

## Patient Conditions:

- For patients on modified diets
- For elderly patients at nutrition risk
- For patients recovering from illness/surgery
- 5 -For patients who need a low-residue diet

## Features:

- Convenient, easy to mix
- Low in saturated fat
- Contains 9 g of total fat and < 5 mg of cholesterol per 10 serving
- High in vitamins and minerals
- For low-cholesterol diets
- Lactose-free, easily digested

15       Ingredients: -D Corn Syrup, Maltodextrin (Corn), Sugar (Sucrose), Corn Oil, Sodium and Calcium Caseinates, Soy Protein Isolate, Artificial Flavor, Potassium Citrate, Magnesium Chloride, Sodium Citrate, Calcium Phosphate Tribasic, Potassium Chloride, Soy Lecithin, Ascorbic Acid, Choline Chloride, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Thiamine Chloride Hydrochloride, Cupric Sulfate, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, 20 Sodium Molybdate, Chromium Chloride, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

25

## Protein:

30       The protein source is a blend of two high-biologic-value proteins: casein and soy.

Sodium and calcium caseinates	84%
Soy protein isolate	16%

## Fat:

The fat source is corn oil.

Corn oil

100%

5

## Carbohydrate:

10 ENSURE POWDER contains a combination of corn syrup, maltodextrin, and sucrose. The mild sweetness of ENSURE POWDER, plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, helps to prevent flavor fatigue and aid in patient compliance.

## Vanilla:

15 Corn Syrup 35%  
Maltodextrin 35%  
Sucrose 30%

## H. ENSURE® PUDDING

20

Usage: ENSURE PUDDING is a nutrient-dense supplement providing balanced nutrition in a nonliquid form to be used with or between meals. It is appropriate for consistency-modified diets (e.g., soft, pureed, or full liquid) or for people with swallowing impairments. ENSURE PUDDING is gluten-free.

## Patient Conditions:

25 -For patients on consistency-modified diets (e.g., soft, pureed, or full liquid)  
30 -For patients with swallowing impairments

## Features:

35 -Rich and creamy, good taste  
-Good source of essential vitamins and minerals  
-Convenient-needs no refrigeration

-Gluten-free

Nutrient Profile per 5 oz: Calories 250, Protein 10.9%,  
Total Fat 34.9%, Carbohydrate 54.2%

5

Ingredients:

Vanilla: -D Nonfat Milk, Water, Sugar (Sucrose),  
Partially Hydrogenated Soybean Oil, Modified Food Starch,  
Magnesium Sulfate, Sodium Stearoyl Lactylate, Sodium  
10 Phosphate Dibasic, Artificial Flavor, Ascorbic Acid, Zinc  
Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate,  
Choline Chloride, Niacinamide, Manganese Sulfate, Calcium  
Pantothenate, FD&C Yellow #5, Potassium Citrate, Cupric  
Sulfate, Vitamin A Palmitate, Thiamine Chloride  
15 Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, FD&C  
Yellow #6, Folic Acid, Biotin, Phylloquinone, Vitamin D3  
and Cyanocobalamin.

20

Protein:

The protein source is nonfat milk.

Nonfat milk

100%

Fat:

25

The fat source is hydrogenated soybean oil.

Hydrogenated soybean oil

100%

Carbohydrate:

30

ENSURE PUDDING contains a combination of sucrose and modified food starch. The mild sweetness and flavor variety (vanilla, chocolate, butterscotch, and tapioca) help prevent flavor fatigue. The product contains 9.2 grams of lactose per serving.

## Vanilla and other nonchocolate flavors:

	Sucrose	56%
5	Lactose	27%
	Modified food starch	17%

## Chocolate:

10	Sucrose	58%
	Lactose	26%
	Modified food starch	16%

## I. ENSURE® WITH FIBER:

15 Usage: ENSURE WITH FIBER is a fiber-containing, nutritionally complete liquid food designed for people who can benefit from increased dietary fiber and nutrients. ENSURE WITH FIBER is suitable for people who do not require a low-residue diet. It can be fed orally or by tube, and can be used as a nutritional supplement to a regular diet or, in appropriate amounts, as a meal replacement. ENSURE WITH FIBER is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.

25 Patient Conditions:

-For patients who can benefit from increased dietary fiber and nutrients

30 Features:

-New advanced formula-low in saturated fat, higher in vitamins and minerals  
-Contains 6 g of total fat and < 5 mg of cholesterol per serving

35 -Rich, creamy taste

- Good source of fiber
- Excellent source of essential vitamins and minerals
- For low-cholesterol diets
- Lactose- and gluten-free

5

Ingredients:

Vanilla: -D Water; Maltodextrin (Corn), Sugar (Sucrose),  
Sodium and Calcium Caseinates, Oat Fiber, High-Oleic  
Safflower Oil, Canola Oil, Soy Protein Isolate, Corn Oil,  
10 Soy Fiber, Calcium Phosphate Tribasic, Magnesium  
Chloride, Potassium Citrate, Cellulose Gel, Soy Lecithin,  
Potassium Phosphate Dibasic, Sodium Citrate, Natural and  
Artificial Flavors, Choline Chloride, Magnesium  
Phosphate, Ascorbic Acid, Cellulose Gum, Potassium  
15 Chloride, Carrageenan, Ferrous Sulfate, Alpha-Tocopheryl  
Acetate, Zinc Sulfate, Niacinamide, Manganese Sulfate,  
Calcium Pantothenate, Cupric Sulfate, Vitamin A  
Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine  
Hydrochloride, Riboflavin, Folic Acid, Chromium Chloride,  
20 Biotin, Sodium Molybdate, Potassium Iodide, Sodium  
Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

Protein:

25 The protein source is a blend of two high-biologic-value proteins-casein and soy.

Sodium and calcium caseinates	80%
Soy protein isolate	20%

30 Fat:

The fat source is a blend of three oils: high-oleic safflower, canola, and corn.

35 High-oleic safflower oil	40%
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Canola oil	40%
Corn oil	20%

5 The level of fat in ENSURE WITH FIBER meets American Heart Association (AHA) guidelines. The 6 grams of fat in ENSURE WITH FIBER represent 22% of the total calories, with 2.01 % of the fat being from saturated fatty acids and 6.7% from polyunsaturated fatty acids. These values are within the AHA guidelines of  $\leq$  30% of total calories from fat,  $<$  10% of the calories from saturated fatty acids, and  $\leq$  10% of total calories from polyunsaturated fatty acids.

10 Carbohydrate: ENSURE WITH FIBER contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla, chocolate, and butter pecan), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

15 20 Vanilla and other nonchocolate flavors:

Maltodextrin	66%
Sucrose	25%
Oat Fiber	7%
Soy Fiber	2%

25 Chocolate:

Maltodextrin	55%
Sucrose	36%
Oat Fiber	7%
Soy Fiber	2%

30 35 Fiber:

The fiber blend used in ENSURE WITH FIBER consists of oat fiber and soy polysaccharide. This blend results in approximately 4 grams of total dietary fiber per 8-fl. oz can. The ratio of insoluble to soluble fiber is 95:5.

5

The various nutritional supplements described above and known to others of skill in the art can be substituted and/or supplemented with the PUFAs produced in accordance with the present invention.

10

#### J.Oxepa™ Nutritional Product

15

Oxepa is a low-carbohydrate, calorically dense, enteral nutritional product designed for the dietary management of patients with or at risk for ARDS. It has a unique combination of ingredients, including a patented oil blend containing eicosapentaenoic acid (EPA from fish oil),  $\gamma$ -linolenic acid (GLA from borage oil), and elevated antioxidant levels.

20

#### Caloric Distribution:

Caloric density is high at 1.5 Cal/mL (355 Cal/8 fl oz), to minimize the volume required to meet energy needs.

The distribution of Calories in Oxepa is shown in Table A.

25

Table A. Caloric Distribution of Oxepa

	per 8 fl oz.	per liter	% of Cal
Calories	355	1,500	---
Fat (g)	22.2	93.7	55.2
Carbohydrate (g)		25	105.528.1
Protein (g)	14.8	62.5	16.7
Water (g)	186	785	---

## Fat:

-Oxepa contains 22.2 g of fat per 8-fl oz serving (93.7 g/L).

5           -The fat source is an oil blend of 31.8% canola oil, 25% medium-chain triglycerides (MCTs), 20% borage oil, 20% fish oil, and 3.2 % soy lecithin. The typical fatty acid profile of Oxepa is shown in Table B.

10           -Oxepa provides a balanced amount of polyunsaturated, monounsaturated, and saturated fatty acids, as shown in Table VI.

15           -Medium-chain triglycerides (MCTs) -- 25% of the fat blend -- aid gastric emptying because they are absorbed by the intestinal tract without emulsification by bile acids.

20           The various fatty acid components of Oxepa™ nutritional product can be substituted and/or supplemented with the PUFAs produced in accordance with this invention.

Table B. Typical Fatty Acid Profile

		% Total	g/8 fl oz*	g/L*
25	Fatty Acids			
	Caproic (6:0)	0.2	0.04	0.18
30	Caprylic (8:0)	14.69	3.1	13.07
	Capric (10:0)	11.06	2.33	9.87
	Palmitic (16:0)	5.59	1.18	4.98
	Palmitoleic	1.82	0.38	1.62
	Stearic	1.94	0.39	1.64
	Oleic	24.44	5.16	21.75
	Linoleic	16.28	3.44	14.49
35	$\alpha$ -Linolenic	3.47	0.73	3.09
	$\gamma$ -Linolenic	4.82	1.02	4.29
	Eicosapentaenoic	5.11	1.08	4.55

	n-3-Docosapentenoic	0.55	0.12	0.49
	Docosahexaenoic	2.27	0.48	2.02
5	Others	7.55	1.52	6.72

Fatty acids equal approximately 95% of total fat.

Table C. Fat Profile of Oxepa.

	% of total calories from fat	55.2
10	Polyunsaturated fatty acids	31.44 g/L
	Monounsaturated fatty acids	25.53 g/L
	Saturated fatty acids	32.38 g/L
	n-6 to n-3 ratio	1.75:1
	Cholesterol	9.49 mg/8 fl oz
15		40.1 mg/L

Carbohydrate:

- The carbohydrate content is 25.0 g per 8-fl-oz serving (105.5 g/L).
- 20 -The carbohydrate sources are 45% maltodextrin (a complex carbohydrate) and 55% sucrose (a simple sugar), both of which are readily digested and absorbed.
- The high-fat and low-carbohydrate content of Oxepa is designed to minimize carbon dioxide (CO<sub>2</sub>) production. High CO<sub>2</sub> levels can complicate weaning in ventilator-dependent patients. The low level of carbohydrate also may be useful for those patients who have developed stress-induced hyperglycemia.
- 25 -Oxepa is lactose-free.

30 Dietary carbohydrate, the amino acids from protein, and the glycerol moiety of fats can be converted to glucose within the body. Throughout this process, the carbohydrate requirements of glucose-dependent tissues (such as the central nervous system and red blood cells) are met. However, a diet free of carbohydrates can lead

to ketosis, excessive catabolism of tissue protein, and loss of fluid and electrolytes. These effects can be prevented by daily ingestion of 50 to 100 g of digestible carbohydrate, if caloric intake is adequate. The 5 carbohydrate level in Oxepa is also sufficient to minimize gluconeogenesis, if energy needs are being met.

Protein:

- Oxepa contains 14.8 g of protein per 8-fl-oz serving (62.5 g/L).
- 10 -The total calorie/nitrogen ratio (150:1) meets the need of stressed patients.
- Oxepa provides enough protein to promote anabolism and the maintenance of lean body mass without precipitating 15 respiratory problems. High protein intakes are a concern in patients with respiratory insufficiency. Although protein has little effect on CO<sub>2</sub> production, a high protein diet will increase ventilatory drive.
- 20 -The protein sources of Oxepa are 86.8% sodium caseinate and 13.2% calcium caseinate.
- The amino acid profile of the protein system in Oxepa meets or surpasses the standard for high quality protein set by the National Academy of Sciences.

25 \* Oxepa is gluten-free.

CLAIMS:

1. An isolated nucleotide sequence or fragment thereof comprising or complementary to a nucleotide sequence encoding a polypeptide having desaturase activity, wherein the amino acid sequence of said polypeptide has at least 50% identity to an amino acid sequence selected from the group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:37, SEQ ID NO:46 and SEQ ID NO:55.
2. An isolated nucleotide sequence or fragment thereof comprising or complementary to a nucleotide sequence having at least 50% identity to a nucleotide sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:36, SEQ ID NO:45 and SEQ ID NO:54.
3. The isolated nucleotide sequence of claim 1 or 2 wherein said sequence encodes a functionally active desaturase which utilizes a monounsaturated or polyunsaturated fatty acid as a substrate.
4. The nucleotide sequence of claim 1 or 2 wherein said sequence is derived from an organism selected from the group consisting of a fungus and an algae.
5. The nucleotide sequence of claim 4 wherein said sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17 and SEQ ID NO:45 is derived from the fungus *Thraustochytrium aureum*, said sequence comprising SEQ ID NO:36 is derived from the fungus *Schizochytrium aggregatum*, and said sequence comprising SEQ ID NO:54 is derived from the algae *Isochrysis galbana*.

6. A purified polypeptide encoded by said nucleotide sequence of claims 1 or 2.

5 7. A purified polypeptide which desaturates polyunsaturated fatty acids at carbon 4 and has an amino acid sequence having at least 50% identity to an amino acid sequence selected from the group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ 10 ID NO:37, SEQ ID NO:46 and SEQ ID NO:55.

8. A method of producing a desaturase comprising the steps of:

15 a) isolating a nucleotide sequence comprising or complementary to a nucleotide sequence:

20 i) encoding a polypeptide having an amino acid sequence having at least 50% identity to an amino acid sequence selected from the group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:37, SEQ ID NO:46 and SEQ ID NO:55 or  
25 ii) having at least 50% identity to a nucleotide sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:36, SEQ ID NO:45 and SEQ ID NO:54;

30 b) constructing a vector comprising: i) said isolated nucleotide sequence operably linked to ii) a promoter;

c) introducing said vector into a host cell for a

time and under conditions sufficient for expression of said desaturase.

9. A vector comprising:

5

a) an isolated nucleotide sequence comprising or complementary to a nucleotide sequence:

10

i) encoding a polypeptide having an amino acid sequence having at least 50% identity to an amino acid sequence selected from the group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:37, SEQ ID NO:46 and SEQ ID NO:55 or

15

ii) having at least 50% identity to a nucleotide sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:36, SEQ ID NO:45 and SEQ ID NO:54, operably linked to

b) a promoter.

20

10. A host cell comprising said vector of claim 9.

25

11. A plant cell, plant or plant tissue comprising said vector of claim 9, wherein expression of said nucleotide sequence of said vector results in production of a polyunsaturated fatty acid by said plant cell, plant or plant tissue.

30

12. The plant cell, plant or plant tissue of claim 11 wherein said polyunsaturated fatty acid is selected from the group consisting of  $\omega 6$ -docosapentaenoic acid and docosahexaenoic acid.

35

13. One or more plant oils or acids expressed by said plant cell, plant or plant tissue of claim 11.

14. A transgenic plant comprising said vector of claim 9, wherein expression of said nucleotide sequence of said vector results in production of a polyunsaturated fatty acid in seeds of said transgenic plant.

5 15. A method for producing a polyunsaturated fatty acid comprising the steps of:

10 a) isolating a nucleotide sequence comprising or complementary to a nucleotide sequence:

15 i) encoding a polypeptide having an amino acid sequence having at least 50% identity to an amino acid sequence selected from the group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:37, SEQ ID NO:46 and SEQ ID NO:55 or

20 ii) having at least 50% identity to a nucleotide sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16 and SEQ ID NO:17, SEQ ID NO:36, SEQ ID NO:45, and SEQ ID NO:54;

25 b) constructing a vector comprising said isolated nucleotide sequence;

c) introducing said vector into a host cell for a time and under conditions sufficient for expression of  $\Delta 4$ -desaturase; and

30 d) exposing said expressed  $\Delta 4$ -desaturase to a substrate polyunsaturated fatty acid in order to convert said substrate to a product polyunsaturated fatty acid.

35 16. The method according to claim 15, wherein said substrate polyunsaturated fatty acid is adrenic acid or  $\omega 3$ -docosapentaenoic acid and said product polyunsaturated fatty acid is  $\omega 6$ -docosapentaenoic acid or docosahexaenoic acid, respectively.

17. The method according to claim 15 further comprising the step of exposing said product polyunsaturated fatty acid to a desaturase in order to convert said product polyunsaturated fatty acid to another polyunsaturated fatty acid.

18. The method according to claim 17 wherein said product polyunsaturated fatty acid is  $\omega 6$ -docosapentaenoic acid and said another polyunsaturated fatty acid is docosahexaenoic acid.

19. A method of producing a polyunsaturated fatty acid comprising the steps of:

15 a) exposing a substrate polyunsaturated fatty acid to one or more enzymes selected from the group consisting of a desaturase and an elongase in order to convert said substrate to a product polyunsaturated fatty acid; and

20 b) exposing said product polyunsaturated fatty acid of step (a) to a  $\Delta 4$ -desaturase comprising an amino acid sequence selected from the group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:37, SEQ ID NO:46 and SEQ ID NO:55, in order to convert 25 said product polyunsaturated fatty acid to a final product polyunsaturated fatty acid.

20. The method of claim 19 wherein said substrate polyunsaturated fatty acid is selected from the group 30 consisting of linoleic acid,  $\gamma$ -linolenic acid, stearidonic acid, arachidonic acid, dihomo- $\gamma$ -linolenic acid, eicosatetraenoic acid, adrenic acid, eicosapentaenoic acid.

21. The method of claim 19 wherein said final product polyunsaturated fatty acid is selected from the group consisting of  $\omega 6$ -docosapentaenoic acid and docosahexaenoic acid.

5

22. A composition comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of 15 and said another polyunsaturated fatty acid produced according to the method of claim 17.

10

23. The composition of claim 22 wherein said product polyunsaturated fatty acid is at least one polyunsaturated fatty acid selected from the group consisting of  $\omega 6$ -docosapentaenoic acid and docosahexaenoic acid.

15

24. The composition of claim 22 wherein said another polyunsaturated fatty acid is docosahexaenoic acid.

20

25. The composition of claim 22 wherein said composition is selected from the group consisting of an infant formula, a dietary supplement and a dietary substitute.

25

26. The composition of claim 22 wherein said composition is administered to a human or an animal.

30

27. The composition of claim 26 wherein said composition is administered enterally or parenterally.

35

28. A method of preventing or treating a condition caused by insufficient intake of polyunsaturated fatty acids comprising administering to said patient said composition

of claim 22 in an amount sufficient to effect said prevention or treatment.

29. An isolated nucleotide sequence or fragment thereof comprising or complementary to a nucleotide sequence encoding a polypeptide having desaturase activity, wherein the amino acid sequence of said polypeptide has at least 30% identity to the amino acid sequence of SEQ ID NO:55.

10

30. An isolated nucleotide sequence or fragment thereof comprising or complementary to a nucleotide sequence having at least 40% identity to the nucleotide sequence of SEQ ID NO:54.

15

31. A purified polypeptide which desaturates polyunsaturated fatty acids at carbon 4 and has an amino acid sequence having at least 30% identity to the amino acid sequence of SEQ ID NO:55.

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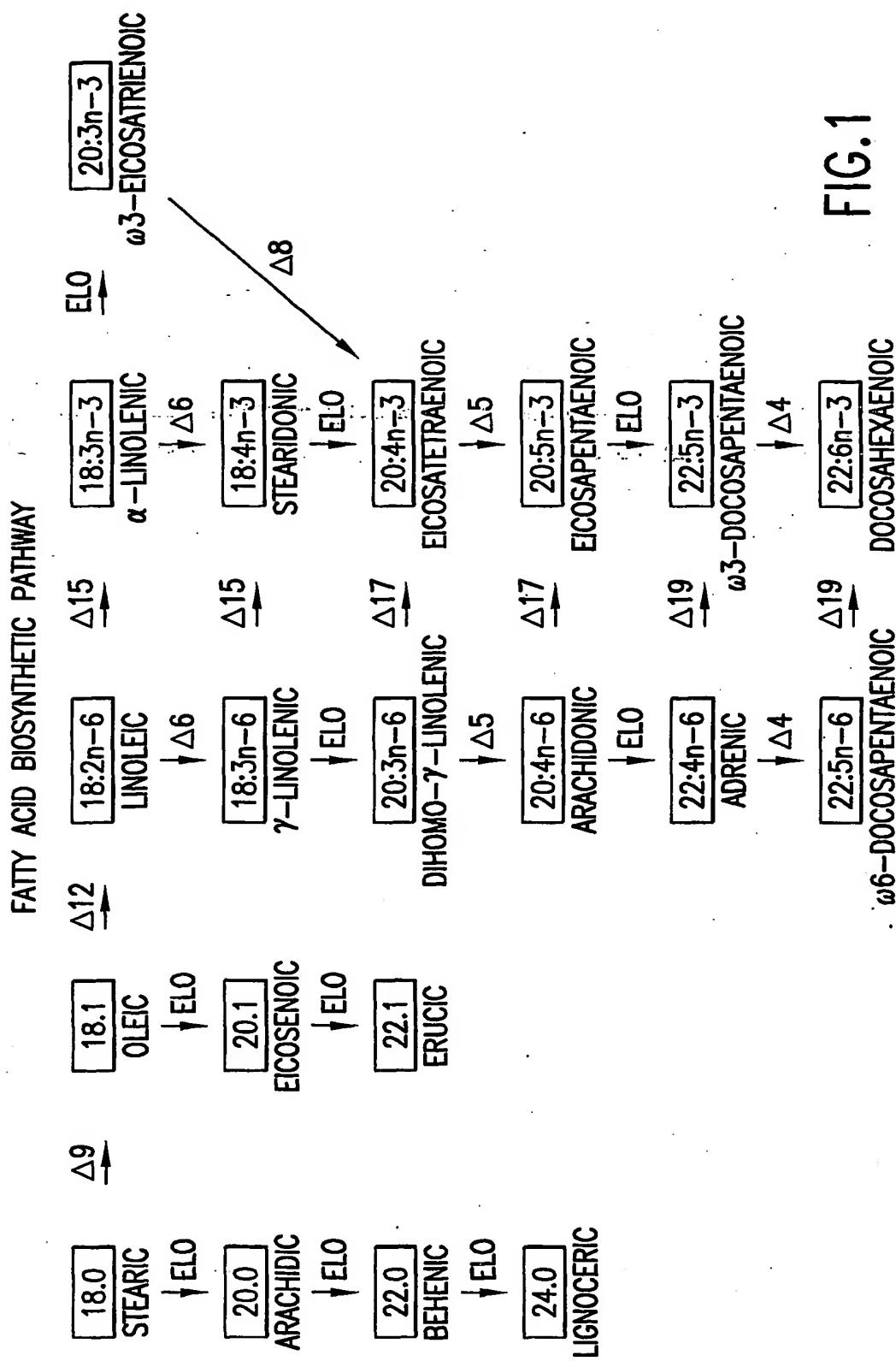


FIG. 1

## Amino Acid Comparison of Delta-4 Desaturases

	1	50			
ppta6	MTVGFDETVT	MDTVRNHNMP	DDAWCAIHGT	VYDITKFSKV	HPGGDIIMLA
ppta8	MTVGFDETVT	MDTVRNHNMP	DDAWCAIHGT	VYDITKFSKV	HPGGDIIMLA
ppta7	MTVGFDETVT	MDTVRNHNMP	DDAWCAIHGT	VYDITKFSKV	HPGGDIIMLA
ppta5	MTVGFDETVT	MDTVRNHNMP	DDAWCAIHGT	VYDITKFSKV	HPGGDIIMLA
	51	100			
ppta6	AGKEATILFE	TYHIKGVPDA	VLRKYKVGKL	PQGKKGETSH	MPTGLD <u>SAY</u>
ppta8	AGKEATILFE	TYHIKGVPDA	VLRKYKVGKL	PQGKKGETSH	MPTGLD <u>SAY</u>
ppta7	AGKEATILFE	TYHIKGVPDA	VLRKYKVGKL	PQGKKGETSH	MPTGLD <u>SAY</u>
ppta5	AGKEATILFE	TYHIKGVPDA	VLRKYKVGKL	PQGKKGETSH	MPTGLD <u>SAY</u>
	101	150			
ppta6	YSWDSEFYRV	LRERVAKKLA	EPGLMQRARM	ELWAKAIFLL	AGFWGSLYAM
ppta8	YSWDSEFYRV	LRERVAKKLA	EPGLMQRARM	ELWAKAIFLL	AGFWGSLYAM
ppta7	YSWDSEFYRV	LRERVAKKLA	EPGLMQRARM	ELWAKAIFLL	AGFWGSLYAM
ppta5	YSWDSEFYRV	LRERVAKKLA	EPGLMQRARM	ELWAKAIFLL	AGFWGSLYAM
	151	200			
ppta6	CVLDPHGGAM	VAAVTLGVFA	AFVGTCIQHD	GSHGAFSKSR	FMNKAAGWTL
ppta8	CVLDPHGGAM	VAAVTLGVFA	AFVGTCIQHD	GSHGAFSKSR	FMNKAAGWTL
ppta7	CVLDPHGGAM	VAAVTLGVFA	AFVGTCIQHD	GSHGAFSKSR	FMNKAAGWTL
ppta5	CVLDPHGGAM	VAAVTLGVFA	AFVGTCIQHD	GSHGAFSKSR	FMNKAAGWTL
	201	250			
ppta6	DMIGASAMTW	EMQHVLGHHP	YTNLIELMENG	LAKVKGADVD	PKKVDQESDP
ppta8	DMIGASAMTW	EMQHVLGHHP	YTNLIELMENG	LAKVKGADVD	PKKVDQESDP
ppta7	DMIGASAMTW	EMQHVLGHHP	YTNLIELMENG	LAKVKGADVD	PKKVDQESDP
ppta5	DMIGASAMTW	EMQHVLGHHP	YTNLIELMENG	LAKVKGADVD	PKKVDQESDP
	251	300			
ppta6	DVFSTYPMLR	LHPWHRQRFY	HKFQHLYAPF	IFGFMTINKV	ISQDVGVVLR
ppta8	DVFSTYPMLR	LHPWHRQRFY	HKFQHLYAPF	IFGSMTINKV	ISQDVGVVLR
ppta7	DVFSTYPMLR	LHPWHRQRFY	HKFQHLYAPL	IFGFMTINKV	ISQDVGVVLR
ppta5	DVFSTYPMLR	LHPWHRQRFY	HKFQHLYAPF	IFGFMTINKV	ISQDVGVVLR

FIG.2A

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## Amino Acid Comparison of Delta-4 Desaturases

	301	350
ppta6	KRLFQIDANC RYGSPWYVAR FWIMKLLTTL YMVALPMYMQ GPAQGLKLFF	
ppta8	KRLFQIDANC RYGSPWYVAR FWIMKLLTTL YMVALPMYMQ GPAQGLKLFF	
ppta7	KRLFQIDANC RYGSPWNVAR FWIMKLLTTL YMVALPMYMQ GPAQGLKLFF	
ppta5	KRLFQIDANC RYGSPWYVAR FWIMKLLTTL YIVALPMYMQ GPAQGLKLFF	
	351	400
ppta6	MAHFTCGEVL ATMFIVNHI EGVSYASKDA VKGVMAPPRT VHGVTPMQVT	
ppta8	MAHFTCGEVL ATMFIVNHI EGVSYASKDA VKGVMAPPRT VHGVTPMQVT	
ppta7	MAHFTCGEVL ATMFIVNHI EGVSYASKDA VKGVMAPPRT VHGVTPMQVT	
ppta5	MAHFTCGEVL ATMFIVNHI EGVSYASKDA VKGVMAPPRT VHGVTPMQVT	
	401	450
ppta6	QKALSAAEST KSDADKTTMI PLNDWAAVQC QTSVNWAVGS WFWNHFSGGL	
ppta8	QKALSAAESA KSDADKTTMI PLNDWAAVQC QTSVNWAVGS WFWNHFSGGL	
ppta7	QKALSAAEST KSDADKTTMI PLNDWAAVQC QTSVNWAVGS WFWNHFSGGL	
ppta5	QKALSAAEST KSDADKTTMI PLNDWAAVQC QTSVNWAVGS WFWNHFSGGL	
	451	500
ppta6	NHQIEHHCFP QNPHTVNVI SGIVKETCEE YGVPYQAEIS LFSAYFKMLS	
ppta8	NHQIEHHCFP QNPHTVNVI SGIVKETCEE YGVPYQAEIS LFSAYFKMLS	
ppta7	NHQIEHHCFP QNPHTVNVI SGIVKETCEE YGVPYQAEIS LFSAYFKMLS	
ppta5	NHQIEHHCFP QNPHTVNVI SGIVKETCEE YGVPYQAEIS LFSAYFKMLS	
	500	
ppta6	HLRTLGNEDL TAWST*	
ppta8	HLRTLGNEDL TAWST*	
ppta7	HLRTLGNEDL TAWST*	
ppta5	HLRTLGNEDL TARST*	

FIG. 2B

Gene Sequence of Delta 4-Desaturase of *Thraustochytrium aureum* (ATCC 34304) from plasmid pRTA5

atgacggctcg	ggtttgacga	aacgggtact	atggacacgg	tccgcaacca	caacatcccg	60
gacggccct	ggtgccgat	ccacggcacc	gtgtacgaca	tcaccaagtt	caggaaagggt	120
caccggcg	gggacatcat	catgtggcc	gctggcaagg	aggccaccat	cctgttgcag	180
acgtaccaca	tcaagggggt	cccgacgcg	gtgtctgcga	agtacaagggt	cgccaaggctc	240
cccaggcca	agaaggggca	aacggccac	atgcccaccc	ggctcgactc	ggccctccatc	300
tactcggtt	acagcggtt	ttacagggtt	ctccgcgagc	gctgcggca	gaagctggcc	360
gaggccggcc	tcatgacgcg	cgcgcgtatg	gagctctggg	ccaaggcgat	cttcctctgt	420
gcagggttct	ggggctccct	ttacgcccatt	tgctgtcttag	accggcacgg	cggtgcccata	480
gtagccggcg	ttacgctcg	cgtgttcgt	gccttttgtcg	gaacttgcata	ccagcaccgac	540
ggcagccacg	gccccttc	caagtgcgca	ttcatgaaaca	agggggggg	ctggaccctc	600
gacatgtatcg	gcccggggc	gtatgacctgg	gatgtgcggc	acgttcttgg	ccaccacccg	660
tacccaacc	tcatcgat	ggagaacggt	ttggccaaagg	tcaaggggcgc	cgacgtcgac	720
ccgaaagg	tcgaccagg	gaggacccgg	gacgtctca	gtacgtaccc	gtgtttcgc	780
ctgaccgg	ggcacccca	gcyttttac	cacaagtcc	agcacctgtt	cgccccgtt	840
atctttgggt	ttatgtacat	taacaagggt	atttcccagg	atgtcgggt	tgtgttgcgc	900
aaggccctgt	tccagatcg	cgcacaactgc	cgttatggca	gccccctggta	cgtggccgc	960
ttctggatca	tgaaggctct	caccacgctc	tacacgggg	cgcttccccat	gtacatgcag	1020
gggcctgct	agggttggaa	gtttttcttc	atggcccaact	tcacctgcgg	agaggtctct	1080
gcaccatgt	ttagtgtcaa	ccacatcatc	gaggggcgtca	gctacgcctc	caaggacgcg	1140
gtcaaggccg	tcatggctcc	gcccgcact	gtgcacgggt	tcaccccgat	gcagggtacg	1200
caaaaggcgc	tcatgtggcc	cgagtgcacc	aagtggacg	ccgacaaagac	gaccatgtatc	1260
ccctcaacg	actggggcgc	tgtgcagtgc	cagacccctgt	tgaacttggc	tgtcggttgc	1320
tggtttggaa	accactttc	ggggggccctc	aaccaccca	tttagccacca	ctgtttcccc	1380
caaaaccccc	acacggtcaa	cgtctacatc	tcgggcatacg	tcaaggagac	ctgcgaagaa	1440
tacggcggtgc	cgtaccaggc	tgagatcaggc	ctctttctgt	cctatttcaa	gatgctgtcg	1500
cacccctccgca	cgctcgccaa	cgaggaccc	acggccaggctc	ccacgttga		1548

FIG. 3

Gene Sequence of Delta 4-Desaturase of *Thraustochytrium aureum* (ATCC 34304) from plasmid pRTA6

atgacggctg	ggtttgcacgaa	aacggtgtact	atggcacacgg	tccgcaaccca	caacatggcc	60
gacgacgcct	ggtgcgcgat	ccacggcacc	gtgtacgaca	tcaccaaggat	cagcaagggt	120
caccceggcg	gggacatcat	catgtggcc	gctggcaagg	aggccaccaat	cctgttgag	180
acgtaccaca	tcaaggcggt	tccggacgca	gtgctgcga	agtacaagggt	cgccaagtc	240
cccaggggca	agaaggcgaa	aacaggccac	atgcccaccc	ggctcgactc	ggccttctac	300
tactcggtgg	acagcgaggat	ttacaggggtg	ctccgcgaggc	gctgtgcggaa	gaagctgtgcc	360
gagccggcc	tcatgcagcg	cgcgcgatg	gagctctggg	ccaaaggcgat	cttcctctgt	420
gcaggtttct	ggggctccct	ttacgcccatt	tgcgtgtctag	accgcacgg	cggtgcattat	480
gtagccggcg	ttacgcitcg	cgtgttcgct	gcctttgtcg	gaacttgtcat	ccagcagac	540
ggcagccacg	gccccttc	caagtgcga	ttcatgaaaca	aggggcgaa	ctggaccctc	600
gacatgatcg	gcccggcg	gatgaccctgg	gagatgcaggc	acgttctgg	ccaccacccg	660
tacaccaacc	tcatcgagat	ggagaacgggt	ttggccaaagg	tcaaggggcgc	cgacgtgcac	720
ccgaaagg	tcgaccaggaa	gaggcacccg	gacgtcttca	gtacgtaccc	gtatgttcgc	780
ctgcacccgt	ggcaccccca	gctgttttac	cacaaggtttac	agcacctgtt	cgccccgttt	840
atctttgggt	ttatgacgat	taacaagggtg	atttccagg	atgtcggggt	tgtgtgcgc	900
aagccctgt	tccagatcg	cgcacaactgc	cgtatggca	gcccttgta	cgtggccgc	960
ttctggatca	tgaagcttct	caccacgctc	tacatgtgg	cgcttcccat	gtacatgcag	1020
gggcctgtct	aggcttgtaa	gctttttcttc	atggccact	tcacccatgtcg	agagggtctc	1080
gccaccatgt	ttatgttcaa	ccacatcatc	gagggcgatc	gtacgttcc	caaggacgc	1140
gtcaaggggcg	tcatggctcc	ggccgcgact	gtgcacggtg	tcaccccgat	gcagggtacg	1200
caaaggcgcc	tcaagtggcc	cgagtcgacc	aagtggacg	ccgacaagac	gaccatgtatc	1260
ccctcaacg	actggccgc	tgtcgagtgc	cagacctctg	tgaactgggc	tgtcggtcg	1320
tggtttggaa	accactttc	ggggggccctc	aaccacaga	ttgagcacca	ctgcttcccc	1380
caaaccccc	acacggtcaa	cgtctacatc	tcaaggatcg	tgcgaagaa	1440	
tacggcg	cgtaccaggc	tgagatcagc	ctcttctgt	cctatttcaa	gtatgtcg	1500
cacccctccgca	cgctcgccaa	cgaggaccc	acggccttgtt	ccacgtga	1548	

FIG. 4

Gene Sequence of Delta 4-Desaturase of *Thraustochytrium aureum* (ATCC 34304) from plasmid pRTA7

atgacggctg	ggtttgcacga	aacggtgact	atggcacacgg	tccgcaaccca	caacatggcc	60
gacgacgcct	ggtgcggat	ccacggcacc	gtgtacgaca	tcaccaagtt	cagcaaggtg	120
caccggcg	gggacatcat	catgtggcc	gctggcaagg	aggccaccat	cctgttcgag	180
acctaccaca	tcaagggcg	ccggacgctg	gtgtcgca	agtacaagggt	cggcaagtc	240
cccaggcg	agaaggcg	aacagccac	atgcccaccc	ggctcgactc	ggcctccatc	300
tactcg	ttacagggtt	ttacagggtt	ctccgcgagc	gctgtggccaa	gaagctggcc	360
gagggcc	tcatgcacg	cgcgcacatg	gagctctggg	ccaaaggcgat	cttcctctgt	420
gcaggtttct	gggctccct	ttacgcccatt	tgcggtctatg	tgcggtctatg	cgggtccatg	480
gtagccgc	ttacgctcg	cgtgttcgt	gcctttgtcg	gaacttgtcat	ccagcacgac	540
ggcagccacg	gcgccttctc	caagtgcgca	ttcatgaaaca	aggggccggg	ctggaccctc	600
gacatgtatcg	gcgcgagcgc	gatgacctgg	gagatgcagc	acgttcttgg	tcaccacccg	660
tacaccaacc	tcatcgatg	ggagaacacgt	ttggccaaagg	tcaaggcg	cgacgtcgac	720
ccgaaagg	tcgaccagg	gaggcacccg	gacgtctca	gtacgtaccc	gtatgttgc	780
ctgaccacgt	ggcaccgc	gcgttttac	cacaagtcc	agcacctgtat	cgccccgtt	840
atctttgggt	ttatgacat	taacaagggt	atttccagg	atgtcggggt	tgtgctgcgc	900
aaggccgt	tccagatcg	cgcacaactgc	cggatggca	gcccctggaa	cgtggccgc	960
ttctggatca	tgaagctct	caccacgctc	tacatgtgg	cgcttccat	gtacatgcag	1020
gggcctgtc	agggttgtaa	gctttcttc	atggccact	tcacccatgt	agaggtctc	1080
gcacccatgt	ttatgttcaa	ccacatcatc	gaggcgatca	gctacgttcc	caaggacgc	1140
gtcaaggcg	tcatggctc	gcccgcact	gtgcacgtgt	tcaccccgat	gcagggtgacg	1200
caaaggcg	tcaagtggc	cgagtcgacc	aagtgcgacg	ccgacaagac	gaccatgtatc	1260
ccctcaacg	actggccgc	tgtgcagtgc	cagacctgt	tgaactggc	tgtcggtcg	1320
tggtttgg	accactttc	ggggggccta	aaccacaga	ttgaggacca	ctgcttcccc	1380
caaaacccc	acacggtcaa	cgtctacatc	tcgggcatcg	tcaaggagac	ctgcgaagaa	1440
tacggcgtgc	cgtacaggc	tgagatcagc	ctcttctgt	cctatttcaa	gatgctgtcg	1500
caccccgca	cgctcgccaa	cgaggacctc	acggcctgtt	ccacgtgtaa		1548

FIG. 5

Gene Sequence of Delta 4-Desaturase of *Thraustochytrium aureum* (ATCC 34304) from plasmid pRTA8

atgacggctg	ggtttgcgaa	aacggtgact	atggcacccgg	tccgcaacca	caacatggcg	60
gacgacgcct	ggtgcgcgat	ccacggcacc	gtgtacgaca	tcaccaagtt	cagaagggtg	120
caccggcg	gggacatcat	catgtggcc	gctggcaagg	aggccaccat	cctgttcgag	180
acctaccaca	tcaaggcggt	cccgaccg	gtgtcgca	agtacaagggt	cgcaagctc	240
cccaggca	agaaggcgaa	aacgagccac	atgcccacccg	ggctcgactc	ggccctccatc	300
tactcggtt	acagcgagg	ttacagggtt	ctccgcgagc	ggtcgccaa	gaagctggcc	360
gagccggcc	tcatgcgacg	cgcgcatg	gagctctgg	ccaaggcgat	cttcctctgt	420
gcaggttct	ggggctccct	ttacgcccatt	tgctgtctgt	acccgcacgg	cggtgcctatg	480
gtagccgg	ttacgctcg	cgtgttcgt	gcctttgtcg	gaacttgcat	ccaggacac	540
ggcagccacg	gcgccttctc	caagtgcga	ttcatgaaca	agggggggg	ctggaccctc	600
gacatgatcg	gcccggatgc	gatgaccctgg	gagatgcagc	acgttcttgg	ccaccacccg	660
tacaccaacc	tcatcgagat	ggagaacgg	ttggcccaagg	tcggcgcgc	cgacgtgcac	720
ccgagaagg	tcgaccagg	gaggacccg	gacgtctca	gtacgtaccc	gtatgttcgc	780
ctgaccgg	ggcaccggca	gctgtttac	cacaagtcc	agcacctgt	cgccccgtt	840
atctttgg	ctatgacgat	taacaagggt	atttccagg	atgtcggtt	tgtgtgcgc	900
aaggccgt	tccagatcg	cgcacaactgc	cggatggca	gccccctggta	cgtggccgc	960
ttcggatca	tgaaggctct	caccacgctc	tacatgggg	cgcttcccat	gtacatgcag	1020
gggcctgtc	agggttggaa	gcttttttttcc	atggccact	tcacctgg	agaggctctc	1080
gccaccatgt	ttatgttcaa	ccacatcatc	cggtatggca	gctacgttcc	caaggacgcg	1140
gtcaaggcg	tcatggctcc	ggcgccact	gtgcacgggt	tcaccccgat	gcaggtgtacg	1200
caaaaggcg	tcaatggcc	cggatggcc	aagtggacg	gaccatgtatc	gaccatgtatc	1260
ccctcaacg	actggggccgc	tgtgcagtgc	cagacccctgt	tgtcggttgc	tgtcggttgc	1320
tggtttgg	accactttc	ggggggccctc	aaccaccaga	ttgaggacca	ctgtttcccc	1380
caaaacccc	acacggtcaa	cgttcatcatc	tcgggcatcg	tcaaggagac	ctgcgaagaa	1440
tacggcg	cgtaccaggc	tgaggatcagc	cttttctgt	cctatttcaa	gatgtgtcg	1500
caccccgca	cgctcgccaa	cgaggaccc	acggccttgtt	ccacgtga		1548

FIG. 6

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Gene Sequence of Delta 4-Desaturase of *Thraustochytrium aureum* (ATCC 34304) from plasmid pRTA5

Met	Thr	Val	Gly	Phe	Asp	Glu	Thr	Val	Thr	Met	Asp	Thr	Val	Arg	Asn
1										10				15	
His	Asn	Met	Pro	Asp	Asp	Ala	Trp	Cys	Ala	Ile	His	Gly	Thr	Val	Tyr
										25				30	
Asp	Ile	Thr	Lys	Phe	Ser	Lys	Val	His	Pro	Gly	Gly	Asp	Ile	Ile	Met
										40			45		
Leu	Ala	Ala	Gly	Lys	Glu	Ala	Thr	Ile	Leu	Phe	Glu	Thr	Tyr	His	Ile
										55			60		
Lys	Gly	Val	Pro	Asp	Ala	Val	Leu	Arg	Lys	Tyr	Lys	Val	Gly	Lys	Leu
65									75					80	
Pro	Gln	Gly	Lys	Lys	Gly	Glu	Thr	Ser	His	Met	Pro	Thr	Gly	Leu	Asp
										85		90		95	
Ser	Ala	Ser	Tyr	Tyr	Ser	Trp	Asp	Ser	Glu	Phe	Tyr	Arg	Val	Leu	Arg
									100		105		110		
Glu	Arg	Val	Ala	Lys	Lys	Leu	Ala	Glu	Pro	Gly	Leu	Met	Gln	Arg	Ala
								115		120		125			
Arg	Met	Glu	Leu	Trp	Ala	Lys	Ala	Ile	Phe	Leu	Leu	Ala	Gly	Phe	Trp
	130					135					140				
Gly	Ser	Leu	Tyr	Ala	Met	Cys	Val	Leu	Asp	Pro	His	Gly	Gly	Ala	Met
145						150					155			160	
Val	Ala	Ala	Val	Thr	Leu	Gly	Val	Phe	Ala	Ala	Phe	Val	Gly	Thr	Cys
								165		170		175			
Ile	Gln	His	Asp	Gly	Ser	His	Gly	Ala	Phe	Ser	Lys	Ser	Arg	Phe	Met
								180		185		190			
Asn	Lys	Ala	Ala	Gly	Trp	Thr	Leu	Asp	Met	Ile	Gly	Ala	Ser	Ala	Met
								195		200		205			
Thr	Trp	Glu	Met	Gln	His	Val	Leu	Gly	His	His	Pro	Tyr	Thr	Asn	Leu
							210		215		220				
Ile	Glu	Met	Glu	Asn	Gly	Leu	Ala	Lys	Val	Lys	Gly	Ala	Asp	Val	Asp
225							230		235			240			
Pro	Lys	Lys	Val	Asp	Gln	Glu	Ser	Asp	Pro	Asp	Val	Phe	Ser	Thr	Tyr
								245		250		255			
Pro	Met	Leu	Arg	Leu	His	Pro	Trp	His	Arg	Gln	Arg	Phe	Tyr	His	Lys
								260		265		270			
Phe	Gln	His	Leu	Tyr	Ala	Pro	Phe	Ile	Phe	Gly	Phe	Met	Thr	Ile	Asn
								275		280		285			
Lys	Val	Ile	Ser	Gln	Asp	Val	Gly	Val	Val	Leu	Arg	Lys	Arg	Leu	Phe
								290		295		300			
Gln	Ile	Asp	Ala	Asn	Cys	Arg	Tyr	Gly	Ser	Pro	Trp	Tyr	Val	Ala	Arg
305								310		315		320			
Phe	Trp	Ile	Met	Lys	Leu	Leu	Thr	Thr	Leu	Tyr	Thr	Val	Ala	Leu	Pro
								325		330		335			
Met	Tyr	Met	Gln	Gly	Pro	Ala	Gln	Gly	Leu	Lys	Leu	Phe	Phe	Met	Ala
								340		345		350			

FIG.7A

His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile Val Asn His  
355 360 365  
Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val  
370 375 380  
Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met Gln Val Thr  
385 390 395 400  
Gln Lys Ala Leu Ser Ala Ala Glu Ser Thr Lys Ser Asp Ala Asp Lys  
405 410 415  
Thr Thr Met Ile Pro Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr  
420 425 430  
Ser Val Asn Trp Ala Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly  
435 440 445  
Gly Leu Asn His Gln Ile Glu His His Cys Phe Pro Gln Asn Pro His  
450 455 460  
Thr Val Asn Val Tyr Ile Ser Gly Ile Val Lys Glu Thr Cys Glu Glu  
465 470 475 480  
Tyr Gly Val Pro Tyr Gln Ala Glu Ile Ser Leu Phe Ser Ala Tyr Phe  
485 490 495  
Lys Met Leu Ser His Leu Arg Thr Leu Gly Asn Glu Asp Leu Thr Ala  
500 505 510  
Arg Ser Thr  
515

FIG. 7B

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Gene Sequence of Delta 4-Desaturase of *Thraustochytrium aureum* (ATCC 34304) from plasmid pRTA6

Met	Thr	Val	Gly	Phe	Asp	Glu	Thr	Val	Thr	Met	Asp	Thr	Val	Arg	Asn
1										10				15	
His	Asn	Met	Pro	Asp	Asp	Ala	Trp	Cys	Ala	Ile	His	Gly	Thr	Val	Tyr
										25				30	
Asp	Ile	Thr	Lys	Phe	Ser	Lys	Val	His	Pro	Gly	Gly	Asp	Ile	Ile	Met
										40			45		
Leu	Ala	Ala	Gly	Lys	Glu	Ala	Thr	Ile	Leu	Phe	Glu	Thr	Tyr	His	Ile
										55			60		
Lys	Gly	Val	Pro	Asp	Ala	Val	Leu	Arg	Lys	Tyr	Lys	Val	Gly	Lys	Leu
										75			80		
Pro	Gln	Gly	Lys	Lys	Gly	Glu	Thr	Ser	His	Met	Pro	Thr	Gly	Leu	Asp
										85			95		
Ser	Ala	Phe	Tyr	Tyr	Ser	Trp	Asp	Ser	Glu	Phe	Tyr	Arg	Val	Leu	Arg
										100			110		
Glu	Arg	Val	Ala	Lys	Lys	Leu	Ala	Glu	Pro	Gly	Leu	Met	Gln	Arg	Ala
										115			125		
Arg	Met	Glu	Leu	Trp	Ala	Lys	Ala	Ile	Phe	Leu	Leu	Ala	Gly	Phe	Trp
										130			140		
Gly	Ser	Leu	Tyr	Ala	Met	Cys	Val	Leu	Asp	Pro	His	Gly	Gly	Ala	Met
										145			155		160
Val	Ala	Ala	Val	Thr	Leu	Gly	Val	Phe	Ala	Ala	Phe	Val	Gly	Thr	Cys
										165			170		175
Ile	Gln	His	Asp	Gly	Ser	His	Gly	Ala	Phe	Ser	Lys	Ser	Arg	Phe	Met
										180			185		190
Asn	Lys	Ala	Ala	Gly	Trp	Thr	Leu	Asp	Met	Ile	Gly	Ala	Ser	Ala	Met
										195			200		205
Thr	Trp	Glu	Met	Gln	His	Val	Leu	Gly	His	His	Pro	Tyr	Thr	Asn	Leu
										210			215		220
Ile	Glu	Met	Glu	Asn	Gly	Leu	Ala	Lys	Val	Lys	Gly	Ala	Asp	Val	Asp
										225			230		240
Pro	Lys	Lys	Val	Asp	Gln	Glu	Ser	Asp	Pro	Asp	Val	Phe	Ser	Thr	Tyr
										245			250		255
Pro	Met	Leu	Arg	Leu	His	Pro	Trp	His	Arg	Gln	Arg	Phe	Tyr	His	Lys
										260			265		270
Phe	Gln	His	Leu	Tyr	Ala	Pro	Phe	Ile	Phe	Gly	Phe	Met	Thr	Ile	Asn
										275			280		285
Lys	Val	Ile	Ser	Gln	Asp	Val	Gly	Val	Val	Leu	Arg	Lys	Arg	Leu	Phe
										290			295		300
Gln	Ile	Asp	Ala	Asn	Cys	Arg	Tyr	Gly	Ser	Pro	Trp	Tyr	Val	Ala	Arg
										305			310		320
Phe	Trp	Ile	Met	Lys	Leu	Leu	Thr	Thr	Leu	Tyr	Met	Val	Ala	Leu	Pro
										325			330		335
Met	Tyr	Met	Gln	Gly	Pro	Ala	Gln	Gly	Leu	Lys	Leu	Phe	Phe	Met	Ala
										340			345		350

FIG.8A

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His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile Val Asn His  
355 360 365  
Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val  
370 375 380  
Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met Gln Val Thr  
385 390 395 400  
Gln Lys Ala Leu Ser Ala Ala Glu Ser Thr Lys Ser Asp Ala Asp Lys  
405 410 415  
Thr Thr Met Ile Pro Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr  
420 425 430  
Ser Val Asn Trp Ala Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly  
435 440 445  
Gly Leu Asn His Gln Ile Glu His His Cys Phe Pro Gln Asn Pro His  
450 455 460  
Thr Val Asn Val Tyr Ile Ser Gly Ile Val Lys Glu Thr Cys Glu Glu  
465 470 475 480  
Tyr Gly Val Pro Tyr Gln Ala Glu Ile Ser Leu Phe Ser Ala Tyr Phe  
485 490 495  
Lys Met Leu Ser His Leu Arg Thr Leu Gly Asn Glu Asp Leu Thr Ala  
500 505 510  
Trp Ser Thr  
515

FIG.8B

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Gene Sequence of Delta 4-Desaturase of *Thraustochytrium aureum* (ATCC 34304) from plasmid pRTA7

Met	Thr	Val	Gly	Phe	Asp	Glu	Thr	Val	Thr	Met	Asp	Thr	Val	Arg	Asn
1									10					15	
His	Asn	Met	Pro	Asp	Asp	Ala	Trp	Cys	Ala	Ile	His	Gly	Thr	Val	Tyr
								20		25				30	
Asp	Ile	Thr	Lys	Phe	Ser	Lys	Val	His	Pro	Gly	Gly	Asp	Ile	Ile	Met
								35		40			45		
Leu	Ala	Ala	Gly	Lys	Glu	Ala	Thr	Ile	Leu	Phe	Glu	Thr	Tyr	His	Ile
								50		55			60		
Lys	Gly	Val	Pro	Asp	Ala	Val	Leu	Arg	Lys	Tyr	Lys	Val	Gly	Lys	Leu
								65		70			75		80
Pro	Gln	Gly	Lys	Lys	Gly	Glu	Thr	Ser	His	Met	Pro	Thr	Gly	Leu	Asp
								85		90			95		
Ser	Ala	Ser	Tyr	Tyr	Ser	Trp	Asp	Ser	Glu	Phe	Tyr	Arg	Val	Leu	Arg
								100		105			110		
Glu	Arg	Val	Ala	Lys	Lys	Leu	Ala	Glu	Pro	Gly	Leu	Met	Gln	Arg	Ala
								115		120			125		
Arg	Met	Glu	Leu	Trp	Ala	Lys	Ala	Ile	Phe	Leu	Leu	Ala	Gly	Phe	Trp
								130		135			140		
Gly	Ser	Leu	Tyr	Ala	Met	Cys	Val	Leu	Asp	Pro	His	Gly	Gly	Ala	Met
								145		150			155		160
Val	Ala	Ala	Val	Thr	Leu	Gly	Val	Phe	Ala	Ala	Phe	Val	Gly	Thr	Cys
								165		170			175		
Ile	Gln	His	Asp	Gly	Ser	His	Gly	Ala	Phe	Ser	Lys	Ser	Arg	Phe	Met
								180		185			190		
Asn	Lys	Ala	Ala	Gly	Trp	Thr	Leu	Asp	Met	Ile	Gly	Ala	Ser	Ala	Met
								195		200			205		
Thr	Trp	Glu	Met	Gln	His	Val	Leu	Gly	His	His	Pro	Tyr	Thr	Asn	Leu
								210		215			220		
Ile	Glu	Met	Glu	Asn	Gly	Leu	Ala	Lys	Val	Lys	Gly	Ala	Asp	Val	Asp
								225		230			235		240
Pro	Lys	Lys	Val	Asp	Gln	Glu	Ser	Asp	Pro	Asp	Val	Phe	Ser	Thr	Tyr
								245		250			255		
Pro	Met	Leu	Arg	Leu	His	Pro	Trp	His	Arg	Gln	Arg	Phe	Tyr	His	Lys
								260		265			270		
Phe	Gln	His	Leu	Tyr	Ala	Pro	Leu	Ile	Phe	Gly	Phe	Met	Thr	Ile	Asn
								275		280			285		
Lys	Val	Ile	Ser	Gln	Asp	Val	Gly	Val	Val	Leu	Arg	Lys	Arg	Leu	Phe
								290		295			300		
Gln	Ile	Asp	Ala	Asn	Cys	Arg	Tyr	Gly	Ser	Pro	Trp	Asn	Val	Ala	Arg
								305		310			315		320
Phe	Trp	Ile	Met	Lys	Leu	Leu	Thr	Thr	Leu	Tyr	Met	Val	Ala	Leu	Pro
								325		330			335		
Met	Tyr	Met	Gln	Gly	Pro	Ala	Gln	Gly	Leu	Lys	Leu	Phe	Phe	Met	Ala
								340		345			350		

FIG.9A

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His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile Val Asn His  
355 360 365  
Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val  
370 375 380  
Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met Gln Val Thr  
385 390 395 400  
Gln Lys Ala Leu Ser Ala Ala Glu Ser Thr Lys Ser Asp Ala Asp Lys  
405 410 415  
Thr Thr Met Ile Pro Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr  
420 425 430  
Ser Val Asn Trp Ala Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly  
435 440 445  
Gly Leu Asn His Gln Ile Glu His His Cys Phe Pro Gln Asn Pro His  
450 455 460  
Thr Val Asn Val Tyr Ile Ser Gly Ile Val Lys Glu Thr Cys Glu Glu  
465 470 475 480  
Tyr Gly Val Pro Tyr Gln Ala Glu Ile Ser Leu Phe Ser Ala Tyr Phe  
485 490 495  
Lys Met Leu Ser His Leu Arg Thr Leu Gly Asn Glu Asp Leu Thr Ala  
500 505 510  
Trp Ser Thr  
515

FIG.9B

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Gene Sequence of Delta 4-Desaturase of *Thraustochytrium aureum* (ATCC 34304) from plasmid pRTA8

Met Thr Val Gly Phe Asp Glu Thr Val Thr Met Asp Thr Val Arg Asn  
 1 5 10 15  
 His Asn Met Pro Asp Asp Ala Trp Cys Ala Ile His Gly Thr Val Tyr  
 20 25 30  
 Asp Ile Thr Lys Phe Ser Lys Val His Pro Gly Gly Asp Ile Ile Met  
 35 40 45  
 Leu Ala Ala Gly Lys Glu Ala Thr Ile Leu Phe Glu Thr Tyr His Ile  
 50 55 60  
 Lys Gly Val Pro Asp Ala Val Leu Arg Lys Tyr Lys Val Gly Lys Leu  
 65 70 75 80  
 Pro Gln Gly Lys Lys Gly Glu Thr Ser His Met Pro Thr Gly Leu Asp  
 85 90 95  
 Ser Ala Ser Tyr Tyr Ser Trp Asp Ser Glu Phe Tyr Arg Val Leu Arg  
 100 105 110  
 Glu Arg Val Ala Lys Lys Leu Ala Glu Pro Gly Leu Met Gln Arg Ala  
 115 120 125  
 Arg Met Glu Leu Trp Ala Lys Ala Ile Phe Leu Leu Ala Gly Phe Trp  
 130 135 140  
 Gly Ser Leu Tyr Ala Met Cys Val Leu Asp Pro His Gly Gly Ala Met  
 145 150 155 160  
 Val Ala Ala Val Thr Leu Gly Val Phe Ala Ala Phe Val Gly Thr Cys  
 165 170 175  
 Ile Gln His Asp Gly Ser His Gly Ala Phe Ser Lys Ser Arg Phe Met  
 180 185 190  
 Asn Lys Ala Ala Gly Trp Thr Leu Asp Met Ile Gly Ala Ser Ala Met  
 195 200 205  
 Thr Trp Glu Met Gln His Val Leu Gly His His Pro Tyr Thr Asn Leu  
 210 215 220  
 Ile Glu Met Glu Asn Gly Leu Ala Lys Val Lys Gly Ala Asp Val Asp  
 225 230 235 240  
 Pro Lys Lys Val Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Thr Tyr  
 245 250 255  
 Pro Met Leu Arg Leu His Pro Trp His Arg Gln Arg Phe Tyr His Lys  
 260 265 270  
 Phe Gln His Leu Tyr Ala Pro Phe Ile Phe Gly Ser Met Thr Ile Asn  
 275 280 285  
 Lys Val Ile Ser Gln Asp Val Gly Val Val Leu Arg Lys Arg Leu Phe  
 290 295 300  
 Gln Ile Asp Ala Asn Cys Arg Tyr Gly Ser Pro Trp Tyr Val Ala Arg  
 305 310 315 320  
 Phe Trp Ile Met Lys Leu Leu Thr Thr Leu Tyr Met Val Ala Leu Pro  
 325 330 335  
 Met Tyr Met Gln Gly Pro Ala Gln Gly Leu Lys Leu Phe Phe Met Ala  
 340 345 350

FIG.10A

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His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile Val Asn His  
355 360 365  
Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val  
370 375 380  
Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met Gln Val Thr  
385 390 395 400  
Gln Lys Ala Leu Ser Ala Ala Glu Ser Ala Lys Ser Asp Ala Asp Lys  
405 410 415  
Thr Thr Met Ile Pro Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr  
420 425 430  
Ser Val Asn Trp Ala Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly  
435 440 445  
Gly Leu Asn His Gln Ile Glu His His Cys Phe Pro Gln Asn Pro His  
450 455 460  
Thr Val Asn Val Tyr Ile Ser Gly Ile Val Lys Glu Thr Cys Glu Glu  
465 470 475 480  
Tyr Gly Val Pro Tyr Gln Ala Glu Ile Ser Leu Phe Ser Ala Tyr Phe  
485 490 495  
Lys Met Leu Ser His Leu Arg Thr Leu Gly Asn Glu Asp Leu Thr Ala  
500 505 510  
Trp Ser Thr  
515

FIG.10B

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SEQUENCE ID NO:1

5' -GTBTAYGAYGTBACCGARTGGGTBAAGCGYCAUCBGGHGGH-3'

SEQUENCE ID NO:2

5' -GGHGCYTCGCGYAACTGGTGGAAAGCAYCAGCAYAACGTBCAYCAY-3'

SEQUENCE ID NO:3

5' -RTGRTGVACGTTRTGCTGRTGCTTCCACCAAGTTRGCGGARGCDCC-3'

SEQUENCE ID NO:4

5' -TTGATRGCTARCTYGRGTRGASAARGGVTGGTAC-3'

SEQUENCE ID NO:5

5' -CATCATCATXGGRAAXARRTGRTG-3'

SEQUENCE ID NO:6

5' -CTACTACTACTACAYCAYACXTAYACXAAY-3'

SEQUENCE ID NO:7

5' -CCCAGTCACGACGTTGTAAAACGACGGCCAG-3'

SEQUENCE ID NO:8

5' -GACGATTAACAAGGTGATTCCCAGGATGTC

SEQUENCE ID NO:9

5' -GACTAACTCGAGTCACGTGGACCAGGCCGTGAGGTCTT

FIG.11A

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SEQUENCE ID NO:10

GACTAACTCGAGTTGACGAGGTTGTATGTTGGCGGTTGCTTG-3'

SEQUENCE ID NO:11

5' -AGCGGATAACAATTACACAGGAAACAGC-3'

SEQUENCE ID NO:12

TGGCTACCGTCGTGCTGGATGCAAGTTCCG-3'

SEQUENCE ID NO:13

5' -CGCATGGAATTATGACGGTCGGTTTGACGAAACGGTG-3'

SEQUENCE ID NO:14

1 ATGACGGTCG GGTTTGACGA AACGGTGACT ATGGACACGG TCCGCAACCA  
51 CAACATGCCG GACGACGCCT GGTGCGCGAT CCACGGCACC GTGTACGACA  
101 TCACCAAGTT CAGCAAGGTG CACCCCGGCG GGGACATCAT CATGCTGGCC  
151 GCTGGCAAGG AGGCCACCAT CCTGTTGAG ACGTACCACA TCAAGGGCGT  
201 CCCGGACGCG GTGCTGCGCA AGTACAAGGT CGGCAAGCTC CCCCAGGGCA  
251 AGAAGGGCGA AACGAGCCAC ATGCCACCG GGCTCGACTC GGCCTCCTAC  
301 TACTCGTGGG ACAGCGAGTT TTACAGGGTG CTCCGCGAGC GCGTCGCCAA  
351 GAAGCTGGCC GAGCCCGGCC TCATGCGAGCG CGCGCGCATG GAGCTCTGGG  
401 CCAAGGCAGT CTTCTCTG GCAGGTTCT GGGGCTCCCT TTACGCCATG  
451 TGCCTGCTAG ACCCGCACCG CGGTGCCATG GTAGCCGCCG TTACGCTCGG  
501 CGTGTTCGCT GCCTTTGTCG GAACTTGCAT CCAGCAGGAC GGCAGCCACG  
551 GCGCCTTCTC CAAGTCGCGA TTCTGAACA AGGCGGGCGGG CTGGACCCCTC  
601 GACATGATCG GCGCGAGCGC GATGACCTGG GAGATGCAGC ACGTTCTGG  
651 CCACCACCCG TACACCAACC TCATCGAGAT GGAGAACGGT TTGGCCAAGG  
701 TCAAGGGCGC CGACGTCGAC CCGAAGAAGG TCGACCAGGA GAGCGACCCG  
751 GACGCTTCA GTACGTACCC GATGCTTCGC CTGCACCCGT GGCACCGCCA  
801 GCGGTTTAC CACAAGTTCC AGCACCTGTA CGCCCCGTTT ATCTTTGGGT  
851 TTATGACGAT TAACAAGGTG ATTTCCAGG ATGTCGGGGT TGTGCTGCGC  
901 AAGCGCCTGT TCCAGATCGA CGCCAACTGC CGGTATGGCA GCCCCTGGTA  
951 CGTGGCCCGC TTCTGGATCA TGAAGCTCCT CACCAACGCTC TACACGGTGG  
1001 CGCTTCCCAT GTACATGCAG GGGCCTGCTC AGGGCTTGAA GCTTTCTTC  
1051 ATGGCCCACT TCACCTGCGG AGAGGTCCCTC GCCACCATGT TTATTGTCAA

FIG.11B

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1101 CCACATCATC GAGGGCGTCA GCTACGCTTC CAAGGACGCG GTCAAGGGCG  
 1151 TCATGGCTCC GCCGCGCACT GTGCACGGTG TCACCCCGAT GCAGGTGACG  
 1201 CAAAAGGCGC TCAGTGCGGC CGAGTCGACC AAGTCGGACG CCGACAAGAC  
 1251 GACCATGATC CCCCTCAACG ACTGGGCCGC TGTGCAGTGC CAGACCTCTG  
 1301 TGAACTGGGC TGTCGGGTG TGTTTTGGA ACCACTTTTC GGGCGGCCCTC  
 1351 AACCAACCAGA TTGAGCACCA CTGCTTCCCC CAAAACCCCC ACACGGTCAA  
 1401 CGTCTACATC TCAGGCATCG TCAAGGAGAC CTGCGAAGAA TACGGCGTGC  
 1451 CGTACCAAGGC TGAGATCAGC CTCTCTCTG CCTATTCAA GATGCTGTCG  
 1501 CACCTCCGCA CGCTCGGCAA CGAGGACCTC ACGGCCAGGT CCACGTGA

SEQUENCE ID NO:15

1 ATGACGGTCG GGTTTGACGA AACGGTGACT ATGGACACGG TCCGCAACCA  
 51 TAACATGCCG GACGACGCC GGTGCGCGAT CCACGGCACC GTGTACGACA  
 101 TCACCAAGTT CAGCAAGGTG CACCCCGGCG GGGACATCAT CATGCTGGCC  
 151 GCTGGCAAGG AGGCCACCAT CCTGTTGAG ACGTACACCA TCAAGGGCGT  
 201 CCCGGACGCG GTGCTGCAGA AGTACAAGGT CGGCAAGCTC CCCCAGGGCA  
 251 AGAAGGGCGA AACGAGCCAC ATGCCAACCG GGCTCGACTC GGCCCTTCTAC  
 301 TACTCGTGGG ACAGCGAGTT TTACAGGGTG CTCCGCGAGC GCGTCGCCAA  
 351 GAAGCTGGCC GAGCCCGGCC TCATGCAGCG CGCGCGCATG GAGCTCTGGG  
 401 CCAAGGCAGT CTTCTCTG GCAGGTTTCT GGGGCTCCCT TTACGCCATG  
 451 TGCCTGCTAG ACCCGCACCG CGGTGCCATG GTAGCCGCCG TTACGCTCGG  
 501 CGTGTTCGCT GCCTTGTG TGAACTTGCAT CCAGCACGAC GGCAGGCCACG  
 551 GCGCCTTCTC CAAGTCGCGA TTCTGAACA AGGCGGGGGG CTGGACCCCTC  
 601 GACATGATCG GCGCGAGCGC GATGACCTGG GAGATGCAGC ACGTTCTTGG  
 651 CCACCAACCG TACACCAACC TCATCGAGAT GGAGAACGGT TTGGCCAAGG  
 701 TCAAGGGCGC CGACGTCGAC CCGAAGAAGG TCGACCAGGA GAGCGACCCCG  
 751 GACGTCTTCA GTACGTACCC GATGCTTCGC CTGCACCCGT GGCACCGCCA  
 801 GCGGTTTAC CACAAGTTCC AGCACCTGTA CGCCCCGTTT ATCTTTGGGT  
 851 TTATGACGAT TAACAAGGTG ATTTCCCAGG ATGTCGGGGT TGTGCTGCGC  
 901 AAGCGCTGT TCCAGATCGA CGCCAAGTC CGGTATGGCA GCCCCTGGTA  
 951 CGTGGCCCGC TTCTGGATCA TGAAGCTCCT CACCAACGCTC TACATGGTGG  
 1001 CGCTTCCCAT GTACATGCAG GGGCCTGCTC AGGGCTTGAA GCTTTCTTC  
 1051 ATGGCCCACT TCACCTGCAG AGAGGTCTCTC GCCACCATGT TTATTGTCAA  
 1101 CCACATCATC GAGGGCGTCA GCTACGCTTC CAAGGACGCG GTCAAGGGCG  
 1151 TCATGGCTCC GCCGCGCACT GTGCACGGTG TCACCCCGAT GCAGGTGACG  
 1201 CAAAAGGCGC TCAGTGCAGC CGAGTCGACC AAGTCGGACG CCGACAAGAC  
 1251 GACCATGATC CCCCTCAACG ACTGGGCCGC TGTGCAGTGC CAGACCTCTG  
 1301 TGAACTGGGC TGTCGGGTG TGTTTTGGA ACCACTTTTC GGGCGGCCCTC  
 1351 AACCAACCAGA TTGAGCACCA CTGCTTCCCC CAAAACCCCC ACACGGTCAA  
 1401 CGTCTACATC TCAGGCATCG TCAAGGAGAC CTGCGAAGAA TACGGCGTGC  
 1451 CGTACCAAGGC TGAGATCAGC CTCTCTCTG CCTATTCAA GATGCTGTCG  
 1501 CACCTCCGCA CGCTCGGCAA CGAGGACCTC ACGGCCCTGGT CCACGTGA

FIG.11C

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## SEQUENCE ID NO:16

1 ATGACGGTCG GGTTTGACGA AACGGTGAATGGACACGG TCCGCAACCA  
 51 CAACATGCCG GACGACGCCT GGTGCGCGAT CCACGGCACC GTGTACGACA  
 101 TCACCAAGTT CAGCAAGGTG CACCCCGGCG GGGACATCAT CATGCTGGCC  
 151 GCTGGCAAGG AGGCCACCAT CCTGTTGAG ACCTACCACA TCAAGGGCGT  
 201 CCCGGACGCG GTGCTGCGCA AGTACAAGGT CGGCAAGCTC CCCCAGGGCA  
 251 AGAAGGGCGA AACGAGCCAC ATGCCAACCG GGCTCGACTC GGCTCCTAC  
 301 TACTCGTGGG ACAGCGAGTT TTACAGGGTG CTCCGCGAGC GCGTCGCCAA  
 351 GAAGCTGGCC GAGCCCGGCC TCATGCAGCG CGCGCGCATG GAGCTCTGGG  
 401 CCAAGGCGAT CTTCTCTTG GCAGGTTCT GGGGCTCCCT TTACGCCATG  
 451 TGCCTGCTAG ACCCGCACCG CGGTGCCATG GTAGCCGCCG TTACGCTCGG  
 501 CGTGTTCGCT GCCTTTGTCG GAACTTGCAT CCAGCACGAC GGCAGCCACG  
 551 GCGCCTCTC CAAGTCGCGA TTCACTGAACA AGGCGGGGGG CTGGACCCCTC  
 601 GACATGATCG GCGCGAGCGC GATGACCTGG GAGATGCAGC ACGTTCTTGG  
 651 TCACCAACCG TACACCAACCTCATCGAGAT GGAGAACGGT TTGGCCAAGG  
 701 TCAAGGGCGC CGACGTCGAC CCGAAGAAGG TCGACCAGGA GAGCGACCCG  
 751 GACGTCTTCA GTACGTACCC GATGCTTCGC CTGACCCGT GGCACCGCCA  
 801 GCGGTTTAC CACAAGTCC AGCACCTGTA CGCCCCGCTT ATCTTTGGGT  
 851 TTATGACGAT TAACAAGGTG ATTTCCCAGG ATGTCGGGGT TGTGCTGCGC  
 901 AAGCGCCTGT TCCAGATCGA CGCCAACCTGC CGGTATGGCA GCCCCTGGAA  
 951 CGTGGCCCGC TTCTGGATCA TGAAGCTCCT CACCACGCTC TACATGGTGG  
 1001 CGCTTCCCAT GTACATGCAG GGGCCTGCTC AGGGCTTGAA GCTTTCTTC  
 1051 ATGGCCCAC TCAACCTGCG AGAGGTCTC GCCACCATGT TTATTGTCAA  
 1101 CCACATCATC GAGGGCGTCA GCTACGCTTC CAAGGACGCG GTCAAGGGCG  
 1151 TCATGGCTCC GCGCGCACT GTGACCGTG TCACCCCGAT GCAGGTGACG  
 1201 CAAAAGGCGC TCAGTGCAGGC CGAGTCGACC AAGTCGGACG CCGACAAGAC  
 1251 GACCATGATC CCCCTCAACG ACTGGGCCGC TGTGCAGTGC CAGACCTCTG  
 1301 TGAACCTGGC TGTCGGGTG TGTTTTGGA ACCACTTTT GGGCGGCCCTA  
 1351 AACCAACCAGA TTGAGCACCA CTGCTTCCCC CAAAACCCCC ACACGGTCAA  
 1401 CGTCTACATC TCGGGCATCG TCAAGGAGAC CTGCGAAGAA TACGGCGTGC  
 1451 CGTACCAAGGC TGAGATCAGC CTCTTCTCTG CCTATTTCAA GATGCTGTCG  
 1501 CACCTCCGCA CGCTCGGCAA CGAGGACCTC ACGGCCTGGT CCACGTGA

## SEQUENCE ID NO:17

1 ATGACGGTCG GGTTTGACGA AACGGTGAATGGACACGG TCCGCAACCA  
 51 CAACATGCCG GACGACGCCT GGTGCGCGAT CCACGGCACC GTGTACGACA  
 101 TCACCAAGTT CAGCAAGGTG CACCCCGGCG GGGACATCAT CATGCTGGCC  
 151 GCTGGCAAGG AGGCCACCAT CCTGTTGAG ACCTACCACA TCAAGGGCGT  
 201 CCCGGACGCG GTGCTGCGCA AGTACAAGGT CGGCAAGCTC CCCCAGGGCA  
 251 AGAAGGGCGA AACGAGCCAC ATGCCAACCG GGCTCGACTC GGCTCCTAC  
 301 TACTCGTGGG ACAGCGAGTT TTACAGGGTG CTCCGCGAGC GCGTCGCCAA  
 351 GAAGCTGGCC GAGCCCGGCC TCATGCAGCG CGCGCGCATG GAGCTCTGGG

FIG.11D

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401 CCAAGGCGAT CTTCCCTCCTG GCAGGTTTCT GGGGCTCCCT TTACGCCATG  
 451 TCGTGCTAG ACCCGCACCG CGGTGCCATG GTAGCCGCCG TTACGCTCGG  
 501 CGTGTTCGCT GCCTTTGTCG GAACTTGCAT CCAGCACGAC GGCAGCCACG  
 551 GCGCCTTCTC CAAGTCGCGA TTCATGAACA AGGCAGGCGGG CTGGACCCCTC  
 601 GACATGATCG GCGCGAGTGC GATGACCTGG GAGATGCAGC ACGTTCTTGG  
 651 CCACCAACCG TACACCAACC TCATCGAGAT GGAGAACGGT TTGGCCAAGG  
 701 TCAAGGGCGC CGACGTCGAC CCGAAGAAGG TCGACCAGGA GAGCGACCCCG  
 751 GACGTCTTCA GTACGTACCC GATGCTTCGC CTGCACCCGT GGCACCGCCA  
 801 GCGGTTTTAC CACAAGTTCG AGCACCTGTA CGCCCCGTTT ATCTTTGGGT  
 851 CTATGACGAT TAACAAGGTG ATTTCCCAGG ATGTCGGGTT TGTGCTGCGC  
 901 AAGCCCTGT TCCAGATCGA CGCCAACTGC CGGTATGGCA GCCCCTGGTA  
 951 CGTGGCCCGC TTCTGGATCA TGAAGCTCCT CACCAACGCTC TACATGGTGG  
 1001 CGCTTCCCAT GTACATGCAG GGGCCTGCTC AGGGCTTGAA GCTTTTCTTC  
 1051 ATGGCCCAC TCAACCTGCGG AGAGGTCTC GCCACCATGT TTATTGTCAA  
 1101 CCACATCATC GAGGGCGTCA GCTACGCTTC CAAGGACGCG GTCAAGGGCG  
 1151 TCATGGCTCC GCGCGCACT GTGCACGGTG TCACCCCGAT GCAGGTGACG  
 1201 CAAAAGGCGC TCAGTGCGGC CGAGTCGGCC AAGTCGGACG CCGACAAGAC  
 1251 GACCATGATC CCCCTCAACG ACTGGGCCGC TGTGCAGTGC CAGACCTCTG  
 1301 TGAACCTGGC TGTCGGGTG TGTTTTGGA ACCACTTTT GGGCGGCCCTC  
 1351 AACCAACCAGA TTGAGCACCA CTGCTTCCCC CAAAACCCCC ACACGGTCAA  
 1401 CGTCTACATC TCGGGCATCG TCAAGGAGAC CTGCGAAGAA TACGGCGTGC  
 1451 CGTACCAAGGC TGAGATCAGC CTCTTCTCTG CCTATTCAA GATGCTGTCG  
 1501 CACCTCCGCA CGCTCGGCAA CGAGGACCTC ACGGCCTGGT CCACGTGA

## SEQUENCE ID NO:18

1 MTVGFDETWT MDTVRNHNMP DDAWCAIHGT VYDITKFSKV HPGGDIIMLA  
 51 AGKEATILFE TYHIKGVPDA VLRKYKVGKL PQGKKGETSH MPTGLDSASY  
 101 YSWDSEFYRV LRERVAKKLA EPGLMQRARM ELWAKAIFL AGFWGSLYAM  
 151 CVLDPHGGAM VAAVTLGVFA AFVGTCIQHD GSHGAFSKSR FMNKAAGWTL  
 201 DMIGASAMTW EMQHVLGHHP YTNLIEMENG LAKVKGADVD PKKVDQESDP  
 251 DVFSTYPMLR LHPWHRQRFY HKFQHLYAPF IFGFMTINKV ISQDVGVVLR  
 301 KRLFQIDANC RYGPSWYVAR FWIMKLLTTL YTVALPMYMQ GPAQQLKLFF  
 351 MAHFTCGEVL ATMFIVNHI EGVSYASKDA VKGVMAPPRT VHGVTPMQVT  
 401 QKALSAEAEST KSDADKTTMI PLNDWAAVQC QT SVNWAWS WFWNHFSGGL  
 451 NHQIEHHCFP QNPHTVNVIYI SGIVKETCEE YGVPYQAEIS LFSAYFKMLS  
 501 HLRTLGNEDL TARST\*

## SEQUENCE ID NO:19

1 MTVGFDETWT MDTVRNHNMP DDAWCAIHGT VYDITKFSKV HPGGDIIMLA  
 51 AGKEATILFE TYHIKGVPDA VLRKYKVGKL PQGKKGETSH MPTGLDSAFY

FIG.11E

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101 YSWDSEFYRV LRERVAKKLA EPGLMQRARM ELWAKAIFLL AGFWGSLYAM  
151 CVLDPHGGAM VAAVTLGVFA AFVGTCIQHD GSHGAFSKSR FMNKAAGWTL  
201 DMIGASAMTW EMQHVLGHHP YTNLiemeng LAKVKGADVD PKKVDQESDP  
251 DVFSTYPMLR LHPWHRQRFY HKFQHLYAPF IFGFMTINKV ISQDVGVVLR  
301 KRLFQIDANC RYGSWYVAR FWIMKLLTTL YMVALPMYMQ GPAQGLKLFF  
351 MAHFTCGEVL ATMFIvnHII EGVSYASKDA VKGVMAPPRT VHGVTPMQVT  
401 QKALSAEST KSDADKTTMI PLNDWAAVQC QTSVNWAVGS WFWNHFSGGL  
451 NHQIEHHCFP QNPHTVNVIYI SGIVKETCEE YGVPYQAEIS LFSAYFKMLS  
501 HLRTLGNEDL TAWST\*

SEQUENCE ID NO:20

1 MTVGFDETVT MDTVRNHNMP DDAWCAIHGT VYDITKFSKV HPGGDIIMLA  
51 AGKEATILFE TYHIKGPDA VLRKYKVGKL PQGKKGETSH MPTGLDSASY  
101 YSWDSEFYRV LRERVAKKLA EPGLMQRARM ELWAKAIFLL AGFWGSLYAM  
151 CVLDPHGGAM VAAVTLGVFA AFVGTCIQHD GSHGAFSKSR FMNKAAGWTL  
201 DMIGASAMTW EMQHVLGHHP YTNLiemeng LAKVKGADVD PKKVDQESDP  
251 DVFSTYPMLR LHPWHRQRFY HKFQHLYAPL IFGFMTINKV ISQDVGVVLR  
301 KRLFQIDANC RYGSWNVAR FWIMKLLTTL YMVALPMYMQ GPAQGLKLFF  
351 MAHFTCGEVL ATMFIvnHII EGVSYASKDA VKGVMAPPRT VHGVTPMQVT  
401 QKALSAEST KSDADKTTMI PLNDWAAVQC QTSVNWAVGS WFWNHFSGGL  
451 NHQIEHHCFP QNPHTVNVIYI SGIVKETCEE YGVPYQAEIS LFSAYFKMLS  
501 HLRTLGNEDL TAWST\*

SEQUENCE ID NO:21

1 MTVGFDETVT MDTVRNHNMP DDAWCAIHGT VYDITKFSKV HPGGDIIMLA  
51 AGKEATILFE TYHIKGPDA VLRKYKVGKL PQGKKGETSH MPTGLDSASY  
101 YSWDSEFYRV LRERVAKKLA EPGLMQRARM ELWAKAIFLL AGFWGSLYAM  
151 CVLDPHGGAM VAAVTLGVFA AFVGTCIQHD GSHGAFSKSR FMNKAAGWTL  
201 DMIGASAMTW EMQHVLGHHP YTNLiemeng LAKVKGADVD PKKVDQESDP  
251 DVFSTYPMLR LHPWHRQRFY HKFQHLYAPF IFGSMTINKV ISQDVGVVLR  
301 KRLFQIDANC RYGSWYVAR FWIMKLLTTL YMVALPMYMQ GPAQGLKLFF  
351 MAHFTCGEVL ATMFIvnHII EGVSYASKDA VKGVMAPPRT VHGVTPMQVT  
401 QKALSAESA KSDADKTTMI PLNDWAAVQC QTSVNWAVGS WFWNHFSGGL  
451 NHQIEHHCFP QNPHTVNVIYI SGIVKETCEE YGVPYQAEIS LFSAYFKMLS  
501 HLRTLGNEDL TAWST\*

FIG.11F

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## SEQUENCE ID NO:22

ATGGAGCAGC	TGAAGGCCCTT	TGATAATGAA	GTCAATGCTT	TCTTGGACAA	CATGTTGGA	60
CCACGAGATT	CTCGAGTTCG	CGGGTGGTTC	CTGCTGGACT	CTTACCTTCC	CACCTTCATC	120
CTCACCATCA	CGTACCTGCT	CTCGATATGG	CTGGGTAACA	AGTACATGAA	GAACAGGCCT	180
GCTCTGCTC	TCAGGGCAT	CCTCACCTTG	TATAACCTCG	CAATCACACT	TCTTCTGCG	240
TATATGCTGG	TGGAGCTCAT	CCTCTCCAGC	TGGGAAGGAG	GTTACAACCTT	GCAGTGTCA	300
AATCTCGACA	GTGCAGGAGA	AGGTGATGTC	CGGGTAGCCA	AGGTCTTGTG	GTGGTACTAC	360
TTCTCCAAAC	TAGTGGAGTT	CCTGGACACG	ATTTTCTTIG	TTCTACGAAA	AAAGACCAAT	420
CAGATCACCT	TCCTTCATGT	CTATCACCAC	GCGTCCATGT	TCAACATCTG	GTGGTGTGTT	480
TTGAACTGGA	TACCTTGTGG	TCAAAGCTTC	TTTGGACCCA	CCCTGAACAG	CTTTATCCAC	540
ATTCTCATGT	ACTCCTACTA	CGGCCCTGTCT	GTGTTCCCGT	CCATGCACAA	GTACCTTGG	600
TGGAAGAAGT	ACCTCACACA	GGCTCAGCTG	GTGCAGTTCG	TACTCACCAC	CACGCACACG	660
CTGAGTGCCG	TGGTGAAGCC	CTGTGGCTTC	CCCTTGGCT	GTCTCATCTT	CCAGTCTTCC	720
TATATGATGA	CGCTGGTCAT	CCTGTTCTTA	AACTTCTATA	TTCAGACATA	CCGGAAAAAG	780
CCAGTGAAGA	AAGAGCTGCA	AGAGAAAGAA	GTGAAGAATG	GTTCCCCCAA	AGCCCACTTA	840
ATTGTGGCTA	ATGGCATGAC	GGACAAGAAG	GCTCAATAA			879

## SEQUENCE ID NO:23

Met	Glu	Gln	Leu	Lys	Ala	Phe	Asp	Asn	Glu	Val	Asn	Ala	Phe	Leu	Asp	
1						5			10					15		
Asn	Met	Phe	Gly	Pro	Arg	Asp	Ser	Arg	Val	Arg	Gly	Trp	Phe	Leu	Leu	
									20			25		30		
Asp	Ser	Tyr	Leu	Pro	Thr	Phe	Ile	Leu	Thr	Ile	Thr	Tyr	Leu	Leu	Ser	
							35		40			45				
Ile	Trp	Leu	Gly	Asn	Lys	Tyr	Met	Lys	Asn	Arg	Pro	Ala	Leu	Ser	Leu	
							50		55			60				
Arg	Gly	Ile	Leu	Thr	Leu	Tyr	Asn	Leu	Ala	Ile	Thr	Leu	Leu	Ser	Ala	
65							65		70			75		80		
Tyr	Met	Leu	Val	Glu	Leu	Ile	Leu	Ser	Ser	Trp	Glu	Gly	Gly	Tyr	Asn	
							85		90			95				
Leu	Gln	Cys	Gln	Asn	Leu	Asp	Ser	Ala	Gly	Glu	Gly	Asp	Val	Arg	Val	
							100		105			110				
Ala	Lys	Val	Leu	Trp	Trp	Tyr	Tyr	Phe	Ser	Lys	Leu	Val	Glu	Phe	Leu	
							115		120			125				
Asp	Thr	Ile	Phe	Phe	Val	Leu	Arg	Lys	Lys	Thr	Asn	Gln	Ile	Thr	Phe	
							130		135			140				
Leu	His	Val	Tyr	His	His	Ala	Ser	Met	Phe	Asn	Ile	Trp	Trp	Cys	Val	
145								145		150			155		160	
Leu	Asn	Trp	Ile	Pro	Cys	Gly	Gln	Ser	Phe	Phe	Gly	Pro	Thr	Leu	Asn	
							165		170			175				
Ser	Phe	Ile	His	Ile	Leu	Met	Tyr	Ser	Tyr	Tyr	Gly	Leu	Ser	Val	Phe	
							180		185			190				

FIG.11G

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Pro Ser Met His Lys Tyr Leu Trp Trp Lys Lys Tyr Leu Thr Gln Ala  
 195 200 205  
 Gln Leu Val Gln Phe Val Leu Thr Ile Thr His Thr Leu Ser Ala Val  
 210 215 220  
 Val Lys Pro Cys Gly Phe Pro Phe Gly Cys Leu Ile Phe Gln Ser Ser  
 225 230 235 240  
 Tyr Met Met Thr Leu Val Ile Leu Phe Leu Asn Phe Tyr Ile Gln Thr  
 245 250 255  
 Tyr Arg Lys Lys Pro Val Lys Lys Glu Leu Gln Glu Lys Glu Val Lys  
 260 265 270  
 Asn Gly Phe Pro Lys Ala His Leu Ile Val Ala Asn Gly Met Thr Asp  
 275 280 285  
 Lys Lys Ala Gln  
 290

## SEQUENCE ID NO:24

ccagtgtgct	ggaattcagg	tactactact	acaccatact	tacacgaacc	tgatcgagat	60
ggagaacggc	acccaaaagg	tcacccacgc	cgacgtcgac	cccaagaagg	ccgaccagga	120
gagcggcccg	gacgtcttca	gcacacctcc	catgctccgt	ctgcacccgt	ggcaccgcaa	180
gcgcttctac	caccgcttcc	agcacctgta	cgcgcccgt	ctcttcgggt	tcatgaccat	240
caacaagggt	atcacccagg	atgtgggagt	tgtccctcagc	aagcgtctgt	ttcagatcga	300
tgccaaactgc	cgttacgcca	gcaagtcgta	cggtgcgcgc	ttctggatca	tgaagctgct	360
caccgtccctc	tacatggtgc	ccctcccccgt	gtacacccag	ggccttgcgt	acgggctcaa	420
gctcttcttc	atcgcccact	tttcgtgcgg	cgagctgctg	gccaccatgt	tcatcgtcaa	480
ccacatcatc	gagggcgtct	cgtacgcctc	caaggactct	gtcaagggca	ccatggcgcc	540
gccgcgcacg	gtgcacggcg	tgaccccgat	gcatgacacc	cgcgacgcgc	tcggcaagga	600
gaaggcagcc	accaagcacg	tgccgctcaa	cgactggcc	gcccgtccagt	gccagacctc	660
ggtcaactgg	tcgatcggtct	cgtggttctg	gaaccacttc	tccggcgggc	tcaaccacca	720
gatcgagcac	cacctttcc	ccatgatgat	gatg			754

## SEQUENCE ID NO:25

Gln	Cys	Ala	Gly	Ile	Gln	Val	Leu	Leu	Leu	His	His	Thr	Tyr	Thr	Asn
1				5			10			15					
Leu	Ile	Glu	Met	Glu	Asn	Gly	Thr	Gln	Lys	Val	Thr	His	Ala	Asp	Val
				20			25			30					
Asp	Pro	Lys	Lys	Ala	Asp	Gln	Glu	Ser	Asp	Pro	Asp	Val	Phe	Ser	Thr
				35			40			45					
Tyr	Pro	Met	Leu	Arg	Leu	His	Pro	Trp	His	Arg	Lys	Arg	Phe	Tyr	His
				50			55			60					

FIG.11H

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Arg Phe Gln His Leu Tyr Ala Pro Leu Leu Phe Gly Phe Met Thr Ile  
65 70 75 80  
Asn Lys Val Ile Thr Gln Asp Val Gly Val Val Leu Ser Lys Arg Leu  
85 90 95  
Phe Gln Ile Asp Ala Asn Cys Arg Tyr Ala Ser Lys Ser Tyr Val Ala  
100 105 110  
Arg Phe Trp Ile Met Lys Leu Leu Thr Val Leu Tyr Met Val Ala Leu  
115 120 125  
Pro Val Tyr Thr Gln Gly Leu Val Asp Gly Leu Lys Leu Phe Phe Ile  
130 135 140  
Ala His Phe Ser Cys Gly Glu Leu Leu Ala Thr Met Phe Ile Val Asn  
145 150 155 160  
His Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ser Val Lys Gly  
165 170 175  
Thr Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met His Asp  
180 185 190  
Thr Arg Asp Ala Leu Gly Lys Glu Lys Ala Ala Thr Lys His Val Pro  
195 200 205  
Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr Ser Val Asn Trp Ser  
210 215 220  
Ile Gly Ser Trp Phe Trp Asn His Phe Ser Gly Gly Leu Asn His Gln  
225 230 235 240  
Ile Glu His His Leu Phe Pro Met Met Met Met  
245 250

FIG.11

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Met Glu Gln Leu Lys Ala Phe Asp Asn Glu Val Asn Ala Phe Leu Asp  
 1 5 10 15  
 Asn Met Phe Gly Pro Arg Asp Ser Arg Val Arg Gly Trp Phe Leu Leu  
 20 25 30  
 Asp Ser Tyr Leu Pro Thr Phe Ile Leu Thr Ile Thr Tyr Leu Leu Ser  
 35 40 45  
 Ile Trp Leu Gly Asn Lys Tyr Met Lys Asn Arg Pro Ala Leu Ser Leu  
 50 55 60  
 Arg Gly Ile Leu Thr Leu Tyr Asn Leu Ala Ile Thr Leu Leu Ser Ala  
 65 70 75 80  
 Tyr Met Leu Val Glu Leu Ile Leu Ser Ser Trp Glu Gly Gly Tyr Asn  
 85 90 95  
 Leu Gln Cys Gln Asn Leu Asp Ser Ala Gly Glu Gly Asp Val Arg Val  
 100 105 110  
 Ala Lys Val Leu Trp Trp Tyr Tyr Phe Ser Lys Leu Val Glu Phe Leu  
 115 120 125  
 Asp Thr Ile Phe Phe Val Leu Arg Lys Lys Thr Asn Gln Ile Thr Phe  
 130 135 140  
 Leu His Val Tyr His His Ala Ser Met Phe Asn Ile Trp Trp Cys Val  
 145 150 155 160  
 Leu Asn Trp Ile Pro Cys Gly Gln Ser Phe Phe Gly Pro Thr Leu Asn  
 165 170 175  
 Ser Phe Ile His Ile Leu Met Tyr Ser Tyr Tyr Gly Leu Ser Val Phe  
 180 185 190  
 Pro Ser Met His Lys Tyr Leu Trp Trp Lys Lys Tyr Leu Thr Gln Ala  
 195 200 205  
 Gln Leu Val Gln Phe Val Leu Thr Ile Thr His Thr Leu Ser Ala Val  
 210 215 220  
 Val Lys Pro Cys Gly Phe Pro Phe Gly Cys Leu Ile Phe Gln Ser Ser  
 225 230 235 240  
 Tyr Met Met Thr Leu Val Ile Leu Phe Leu Asn Phe Tyr Ile Gln Thr  
 245 250 255  
 Tyr Arg Lys Lys Pro Val Lys Lys Glu Leu Gln Glu Lys Glu Val Lys  
 260 265 270  
 Asn Gly Phe Pro Lys Ala His Leu Ile Val Ala Asn Gly Met Thr Asp  
 275 280 285  
 Lys Lys Ala Gln  
 290

FIG.12

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ccagtgtgct	ggaattcagg	tactactact	acaccatact	tacacgaacc	tgatcgagat	60
ggagaacggc	acccaaaagg	tcacccacgc	cgacgtcgac	cccaagaagg	ccgaccagga	120
gagcgacccg	gacgtttca	gcacctaccc	catgctccgt	ctgcacccgt	ggcacccgaa	180
gcgccttctac	caccgcttcc	agcacctgta	cgcgcgcgtg	ctcttcgggtt	tcatgaccat	240
caacaaggtg	atcacccagg	atgtgggagt	tgtcctcagc	aagcgtctgt	ttcagatcga	300
tgccaactgc	cgttacgcca	gcaagtcgta	cgttgcgcgc	ttctggatca	tgaagctgct	360
caccgtcctc	tacatggtcg	ccctccccgt	gtacacccag	ggccttgcgtc	acgggctcaa	420
gctttcttc	atcgcccaact	tttcgtgcgg	cgagctgctg	gccaccatgt	tcatcgtaa	480
ccacatcatc	gagggcgtct	cgtacgcctc	caaggactct	gtcaaggggca	ccatggcgcc	540
gccgcgcacg	gtgcacggcg	tgaccccgat	gcatgacacc	cgcgcgcgc	tcggcaagga	600
gaaggcagcc	accaaggacag	tgccgctcaa	cgactggcc	gcggtccagt	gccagacctc	660
ggtcaactgg	tcgatcggt	cgtggttctg	gaaccacttc	tccggcgggc	tcaaccacca	720
gatcgagcac	cacctttcc	ccatgatgat	gatg			754

**FIG. 13**

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Gln Cys Ala Gly Ile Gln Val Leu Leu Leu His His Thr Tyr Thr Asn  
1 5 10 15  
Leu Ile Glu Met Glu Asn Gly Thr Gln Lys Val Thr His Ala Asp Val  
20 25 30  
Asp Pro Lys Lys Ala Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Thr  
35 40 45  
Tyr Pro Met Leu Arg Leu His Pro Trp His Arg Lys Arg Phe Tyr His  
50 55 60  
Arg Phe Gln His Leu Tyr Ala Pro Leu Leu Phe Gly Phe Met Thr Ile  
65 70 75 80  
Asn Lys Val Ile Thr Gln Asp Val Gly Val Val Leu Ser Lys Arg Leu  
85 90 95  
Phe Gln Ile Asp Ala Asn Cys Arg Tyr Ala Ser Lys Ser Tyr Val Ala  
100 105 110  
Arg Phe Trp Ile Met Lys Leu Leu Thr Val Leu Tyr Met Val Ala Leu  
115 120 125  
Pro Val Tyr Thr Gln Gly Leu Val Asp Gly Leu Lys Leu Phe Phe Ile  
130 135 140  
Ala His Phe Ser Cys Gly Glu Leu Leu Ala Thr Met Phe Ile Val Asn  
145 150 155 160  
His Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ser Val Lys Gly  
165 170 175  
Thr Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met His Asp  
180 185 190  
Thr Arg Asp Ala Leu Gly Lys Glu Lys Ala Ala Thr Lys His Val Pro  
195 200 205  
Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr Ser Val Asn Trp Ser  
210 215 220  
Ile Gly Ser Trp Phe Trp Asn His Phe Ser Gly Gly Leu Asn His Gln  
225 230 235 240  
Ile Glu His His Leu Phe Pro Met Met Met  
245 250

FIG.14

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SCORES Initl: 1077 Initn: 1365 Pot: 1371  
>/amd\_mnt/home/thurmond/tacclone/prta-7.pep (516 aa)  
initn: 1365 initl: 1077 opt: 1371  
Smith-Waterman score:1371; 79.1% identity in 249 aa overlap  
(5-247:212-460)

	10	20	30			
saa.pep	QCAGTQVLLHHTYTNLIEMENG	TQKVTHADVDP				
ppta-7.pep	SHGAFSKSRFMNKAAGWTLDMIGASAMTWEMQHVLGHHPYTNLIEMENG	LAKVKGADVDP				
	190	200	210			
	220	230	240			
	40	50	60	70	80	90
saa.pep	KKADQESDPDVFSTYPMRLH	WHRKR	FYHRFQHLYA	PLLFG	MTINKV	ITQDVGVVLSK
ppta-7.pep	KKVDQESDPDVFSTYPMRLH	WHRQR	FYHFKFQHLYA	PLIFG	MTINKV	ISQDVGVVLSK
	250	260	270	280	290	300
	100	110	120	130	140	150
saa.pep	RLFQIDANC	RYASK	SYVARFWIM	KLLTV	LYMVALP	VYTQGLVDGLKLFFIAH
ppta-7.pep	RLFQIDANC	RYGS	SKSYVARFWIM	KLLT	LYMVALPMY	TQGLVDGLKLFFMAH
	310	320	330	340	350	360
	160	170	180	190	200	
saa.pep	TMFIVNH	IIEGVSYASK	DSVKGT	MAPPRTVHG	VTPMHD	TRDALGKEAA-----TKHVP
ppta-7.pep	TMFIVNH	IIEGVSYASK	DAVKGV	MAPPRTVHG	VTPMQVTQK	ALSAE
	370	380	390	400	410	420
	210	220	230	240	250	
saa.pep	LNDWAAVQC	QCTS	VNW	SIGSWF	NHFS	GGLNHQIEHHLFPM
ppta-7.pep	LNDWAAVQC	QCTS	VNW	AVGSWF	NHFS	GGLNHQIEHHCFPQN
	430	440	450	460	470	480
ppta-7.pep	GVPYQAEISL	FSAYFKML	SHLRTLG	NEDLT	AWSTX	
	490	500	510			

FIG. 15

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1 ATGACGGTGG GCGGCGATGA GGTGTACAGC ATGGCGCAGG TGCAGCGACCA  
51 CAACACCCCG GACGACGCCT GGTGCGCCAT CCACGGCGAG GTGTACGAGC  
101 TGACCAAGTT CGCCCGCACC CACCCCGGGG GGGACATCAT CTTGCTGGCC  
151 GCCGGCAAGG AGGCCACCAT CCTGTTGAG ACGTACCAACG TGCAGCCCCAT  
201 CTCCGACGCG GTCCTGCGCA AGTACCGCAT CGGCAAGCTC GCCGCCGCCG  
251 GCAAGGATGA GCCGGCCAAC GACAGCACCT ACTACAGCTG GGACAGCGAC  
301 TTTTACAAGG TGCTCCGCCA GCGTGTCTG GCGCGCTCG AGGAGCGCAA  
351 GATCGCCCGC CGCGCGGGCC CCGAGATCTG GATCAAGGCC GCCATCTCG  
401 TCAGCGGCTT CTGGTCCATG CTCTACCTCA TGTGCAACCTT GGACCCGAAC  
451 CGCGGCGCCA TCCTGGCCGC CATCGCGCTG GGCATCGTCG CGCGCTTCGT  
501 CGGCACGTGC ATTCAAGCACG ACAGCAACCA CGGCACGTTC GCCTTCTCTC  
551 CGTTCATGAA CAAGCTCTCT GGCTGGACGC TCGACATGAT CGGCGCCAGT  
601 GCCATGACCT GGGAGATGCA GCACGTGCTG GGCCACCAAC CGTACACCAA  
651 CCTGATCGAG ATGGAGAACG GCACCCAAAA GGTACCCAC GCCGACGTCG  
701 ACCCCAAGAA GGCGGACCAAG GAGAGCGACC CGGACGTCTT CAGCACCTAC  
751 CCCATGCTCC GTCTGCACCC GTGGCACCGC AAGCGCTTCT ACCACCGCTT  
801 CCAGCACCTG TACCGGCCGC TGCTCTTCGG TTTCATGACC ATCAACAAAGG  
851 TGATCACCCA GGATGTGGGA GTTGTCCCTA GCAAGCGTCT GTTTCAGATC  
901 GATGCCAACT GCCGTTACGC CAGCAAGTCG TACGTTGCGC GCTTCTGGAT  
951 CATGAAGCTG CTCACCGTCC TCTACATGGT CGCCCTCCCC GTGTACACCC  
1001 AGGGCCTTGT CGACGGGCTC AAGCTCTTCT TCATCGCCCA CTTTCTGTGC  
1051 GGCGAGCTGC TGGCCACCAT GTTCATCGTC AACCACATCA TCGAGGGCGT  
1101 CTCGTACGCC TCCAAGGACT CTGTCAAGGG CACCATGGCG CCGCCGCGCA  
1151 CGGTGCACGG CGTGACCCCG ATGCATGACA CCCGCGACGC GCTCGGCAAG  
1201 GAGAAGGCAG CCACCAAGCA CGTGCCGCTC AACGACTGGG CCGCGGTCCA  
1251 GTGCCAGACC TCGGTCAACT GGTCGATCGG CTCGTGGTTC TGGAACCACT  
1301 TCTCCGGCGG GCTCAACAC CAGATCGAGC ACCACCTCTT CCCCAGGCTC  
1351 ACCCACACCA CCTACGTGA CATTCAAGGAT GTGGTGCAGG CGACGTGCGC  
1401 CGAGTACGGG GTCCCGTACC AGTCGGAGCA GAGCCTCTTC TCCGCCTACT  
1451 TCAAGATGCT CTCCACCTT CGGGCGCTCG GCAACGAGCC GATGCCCTCG  
1501 TGGGAGAAGG ACCACCCCAA GTCCAAGTGA

FIG. 16

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1 MTVGGDEVYS MAQVRDHNTP DDAWCAIHGE VYELTKFART HPGGDIILLA  
51 AGKEATILFE TYHVRPISDA VLRKYRIGKL AAAGKDEPAN DSTYYSWDSD  
101 FYKVLRQRVV ARLEERKiar RGGPEIWIKA AILVSGFWSM LYLMCTLDPN  
151 RGAILAAIAL GIVAAFGVTC IQHDGNHGAF AFSPFMNCLS GWTLDLIGAS  
201 AMTWEMQHVL GHHPYTNLIE MENGTQKVTH ADVDPKKADQ ESDPDVFSTY  
251 PMLRLHPWHR KRFYHRFQHL YAPLLFGFMT INKVITQDVG VVLSKRLFQI  
301 DANCYRASKS YVARFWIMKL LTVLYMVALP VYTQGLVDGL KLFFIAHFSC  
351 GELLATMFIV NHIEGVSYA SKDSVKGTMA PPRTVHGVTp MHDTRDALGK  
401 EKAATKHVPL NDWAAVQCQT SVNWSIGSWF WNHFSGGLNH QIEHHLFPL  
451 THHTYVYIQD VVQATCAEYG VPYQSEQSLF SAYFKMLSHL RALGNEPMPS  
501 WEKDHPKSK

**FIG. 17**

1 ATGACGGCCG GATTGAAGA AGTGATCACC ATGAAGCAGG TGAAGGACCG  
51 GAATACGCCG GACGATGCGT GGTGCGTGGT GCATGGCAAG GTGTACGACA  
101 TCACCAAGTT CAAGAACGCT CACCCCGGTG GAGATATAAT CATGTTGGCG  
151 GCTGGCAAGG ACGCCACCAT CCTGTTCGAG ACTTACCAACA TCCGCGGTGT  
201 GCCCGATGCC GTGTTGCGCA AGTATCAGAT CGGCAAACCTT CGGGACGGAA  
251 AGAACAAAGA GGGCGGCAAC GGCCTCGATA GCGCCTCGTA CTACTCCTGG  
301 GACAGCGAGT TTTACCGCGT CCTTCGCGAG CGCGTCTTGA AGCGCTGAA  
351 CGAGCTCAAG CTGTCCAGAC GCGGAGGCTT CGAGATTTGG GCCAAGGCTA  
401 TCTTCTCTTT GACCGGGCTTC TGGTCTTGC TCTACCTCAT GTGCACACTC  
451 AACCCAAATG GGCTTGCAT TCCTGCCGCC ATGATGTTGG GAATCTTGC  
501 TGCCCTCGTA GGAACCTGCA TTCAAGCACGA CGGGAATCAC GGTGCGTTCG  
551 CCCAATCTTC GTGGCTTAAC AAGGCCGCTG GTTGGACTTT GGACATGATT  
601 GGATCCAGCG CCATGACCTG GGAGATGCAG CACGTGCTTG GACATCATCC  
651 GTACACCAAC TTGATTGAAA TGGAGAATGG CAATCAAAAG GTCTCCGGCA  
701 AGCCTGTTGA CACCAAGACT GTCGACCAAG AGAGCGACCC TGATGTCTTT  
751 AGCACCTACC CTATGCTTCG CCTTCACCCCT TGGCACAGCA AAAAGTGGTA  
801 CCACAAATAC CAGCACATCT ATGACCCATT CATCTTGGG TTCATGACCA  
851 TCAACAAAGGT CATTGCACAG GACGTTGGCG TTATCACACG CAAGCGTCTC  
901 TTCCAGATTG ACGCCAACCTG CCGCTACGCT TCTCCGACTT ACGTGCTCG  
951 CTTCTGGATC ATGAAGGTTTC TTACCGTTCT CTACATGGTT GGCCTGCCAA  
1001 TGTACATGCA AGGTCCATGG GAGGGTCTCA AGTTGTTCTT TATTGCGCAC  
1051 TTTACTTGC GCGAGCTGCT GGCCACAATG TTCACTCGTAA ACCACATCAT  
1101 CGAGGGTGTCA AGCTACGCAA GCAAAGATGC CATCAAGGGC GAGATGGCTC  
1151 CACCGAAAAC GGTCCGCGGT GTCACCCCAA TGACGAGAC GCAAAAGGTT  
1201 CTCGACCAAGC GCGAGAAAAGA CATGGACGAA ACTTCTAAGA AGAGCCGCAT  
1251 CCCTCTCAAC GACTGGGCCG CTGTACAGTG CCAGACCACC GTGAACTGGG  
1301 CTATCGGTTTC TTGGTTCTGG AACCACTTTT CGGGGGGCCT CAATCATCAG  
1351 ATTGAGGCATC ATCTGTTCCC CGGCTTGAAT CACACCCACCT ATGTTCACTT  
1401 TCACGATGTG GTCAAAGATA CTTGCGCTGA GTACGGGGTT CCATACCAAGC  
1451 ACGAGGGAGAG TCTATACACT GCCTACTTTA AGATGTTGAA TCATCTCAAG  
1501 ACCCTAGGCA ACGAGCCAAT GCCTGCCTGG GACAAGAACT AA

1 MTAGFEEVIT MKQVKDRNTP DDAWCVWHGK VYDITKFKNA HPGGDIIMLA  
51 AGKDATILFE TYHIRGVPA VLRKYQIGKL PDGKNKEGGN GLDSASYYSW  
101 DSEFYRVLRE RVLKRLNELK LSRRGGFEIW AKAIFLLTGF WSCLYLMCTL  
151 NPNGLAIPAA MMLGIFAAAFV GTCIQHDGNH GAFAQSSWLN KAAGWTLDMI  
201 GSSAMTWEMQ HVLGHHPYTN LIEMENGNQK VSGKPVDTKT VDQESDPDVF  
251 STYPMLRLHP WHSKKWHKY QHIYAPFIFG FMTINKVIAQ DVGVITRKRL  
301 FQIDANCRYA SPTYVARFWI MKVLTLYMV GLPMYMQGPW EGLKLFFIAH  
351 FTCGEELLATM FIVNHHIEGV SYASKDAIKG EMAPPKTVRG VTPMHETQKV  
401 LDQREKDMDE TSKKSRIPLN DWAAVQCQTT VNWAIGSWFW NHFSGGLNHQ  
451 IEHHLFPGLT HTTYVHFHDV VKDTCAEYGV PYQHEESLYT AYFKMLNHLK  
501 TLGNEPMPAW DKN

FIG. 19

DNA sequence of the Delta 4 desaturase gene (pRIG6) from  
Isochrysis galbana (CCMP 1323)

1 ATGTGCAACG CGGCGCAGGT CGAGACGGAG GCCTTGCAGCG CCAAGGAGGC  
51 GGCAAAACCG ACGTGGACGA AGATTCAATGG GCGCACAGTC GACGTGGAGA  
101 CGTTCCGCCA CCCAGGCGGC AACATCCTCG ATTGTTCCCT GGGCATGGA  
151 GCCACAACGT CCTTGAGAC GTTCCACGGT CACCACAAGG GAGCATGGAA  
201 GATGCTCAAG ACGCTGCCCG AGAAGGAGGT CGCCGCCGCC GACATTCCCG  
251 CGCAGAAGGA GGAGCACGTG GCCGAGATGA CACGCCCTCAT GGCCTCATGG  
301 CGCGAGCGCG GGCTGTTCAA GCCGCGTCCC GTGCCCTCAT CCATCTATGG  
351 CCTGTGCGTG ATCTTCGCCA TCGCGGCATC GGTGCGGTGC GCTCCGTACG  
401 CGCCAGTGT GGCTGGCATC GCGGTGGGCA CCTGCTGGGC TCAGTGCAGG  
451 TTCTTGACG ACATGGGCGG CCACCGGGAG TGGGGGCGCA CTTGGTCGTT  
501 TGCCTTCAG CATCTGTTTG AAGGCCTGCT CAAGGGCGGC TCGGCCTCGT  
551 GGTGGCGCAA CCGCCACAAAC AAGCACCATG CCAAGACCAA CGTGCTCGGC  
601 GAGGACGGCG ACCTGCGCAC CACACCCCTTC TTCGCATGGG ACCCTACTCT  
651 GGCCAAGAAA GTGCCCGACT GGTCTCTGCG CACGCAAGGCC TTCACCTTTC  
701 TGCCAGCACT GGGAGCTTAC GTCTTCGTCT TTGCCTTCAC GGTACGCAAG  
751 TACAGTGTGG TGAAGCGTCT CTGGCACGAG GTCGCCCTGA TGGTGGCCCA  
801 CTACGCTCTC TTTTCCTGGG CGCTCAGCGC CGCCGGCGCC TCCCTCAGCT  
851 CGGGCCTCAC CTTCTATTGC ACCGGGTACG CCTGGCAGGG CATCTACCTC  
901 GGCTTCTTCT TCGGCCTATC GCACTTTGCG GTGGAGCGCG TGCCGTCGAC  
951 CGCCACCTGG CTCGAGTCGA CGATGATGGG CACCGTTGAC TGGGGCGGCT  
1001 CCTCCGCCTT CTGCGGCTAC CTCTCCGGCT TCCTCAATAT CCAGATCGAG  
1051 CACCAACATGG CTCCACAAAT GCCAATGGAG AACCTGCGCC AGATCCGGC  
1101 CGACTGCAAG GCCGCGGCCA ACAAGTTGG GCTGCCGTAC CGCGAGCTGA  
1151 CATTGCTCGC GGCGACCAAG CTCATGATGA GCGGCCCTTA CGGGACCGGC  
1201 AAGGACGAGC TCAAGCTGCG CGCGGACCGC CGCAAGTTCA CGAGGGCACA  
1251 GGCGTACATG GGCGCCGCCA GCGCTTTGGT CGACACGCTC AAGGCGGACT  
1301 AA

FIG.20

Amino acid sequence of the Delta 4 desaturase gene (pRIG6)  
from Isochrysis galbana (CCMP 1323)

1 MCNAAQVETQ ALRAKEAAKP TWTKIHGRTV DVETFRHPGG NILDLFQMD  
51 ATTAFETFHG HHKGAWKMLK TLPEKEVAAA DIPAQKEEHV AEMTRLMASW  
101 RERGLFKPRP VASSIYGLCV IFAIAASVAC APYAPVLAGI AVGTCWAQCG  
151 FLQHMGGHRE WGRTWSFAFQ HLFEGLLKGG SASWWRNRHN KHHAKTNVLG  
201 EDGDLRTTPF FAWDPTLAKK VPDWSLRTQA FTFLPALGAY VFVFAFTVRK  
251 YSVVKRLWHE VALMVAHYAL FSWALSAAGA SLSSGLTFYC TGYAWQGIYL  
301 GFFFGLSHFA VERVPSTATW LESTMMGTVD WGGSSAFCGY LSGFLNIQIE  
351 HHMAPQMPME NLRQIRADCK AAAHKFGLPY RELTFVAATK LMMSGLYRTG  
401 KDELKLRADR RKFTRAQAYM GAASALVDTL KAD\*

**FIG.21**

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Comparative analysis of the Delta 4 desaturase from *I. galbana* (CCMP 1323) (pRIG6.pep) and the Delta 4 desaturase from *T. aureum* (ATCC 34304) (pRTA7.pep)

Quality: 68  
Ratio: 0.157  
Percent Similarity: 38.384

Length: 554  
Gaps: 22  
Percent Identity: 30.808

Match display thresholds for the alignment(s):

| = IDENTITY  
: = 2  
: = 1

pRIG6.pepx prta7.pep

FIG. 22A

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310 ..... AVERVPSTA ..... TWLESTMMGTVDW 331  
387 PPRTVHGVTPMQVTQKALSAAESTKSDADKTTMIPNLDWA  
AVQCQTSVNW 436  
332 GGSSAFCGYLSGFLNIQIEHHMAPQMPMENLRQIRADCKAAAHK  
FGLPYR 381  
437 AVGSWFWNHFSGGLNHQIEHHCFPQNPHTVNVYISGIVK  
ETCEEYGVPYQ 486  
382 .ELTFVAATKLMMMSGLYRTGKDELKLRADRRKFTRAQAY  
MGAASALVDTL 430  
487 AEISLFSAYFKMLSHLRTLGNEDLTAWST\*..... 516

FIG.22B

## SEQUENCE LISTING

<110> Abbott Laboratories  
Mukerji, Pradip  
Thurmond, Jennifer M.  
Huang, Yung-Sheng  
Das, Tapas  
Leonard, Amanda E.  
Pereira, Suzette L.

<120> DELTA 4-DESATURASE GENES AND USES  
THEREOF

<130> 6804.US.P1

<140> Not Yet Assigned  
<141> 2002-04-11

<150> US 09/849,199

<151> 2001-05-04

<160> 73

<170> FastSEQ for Windows Version 4.0

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<211> 42  
<212> DNA  
<213> Artificial Sequence

<220>

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<221> misc\_feature  
<222> (3)...(3)  
<223> b = g or c or t/u at position 3

<221> misc\_feature  
<222> (6)...(6)  
<223> y = t/u or c at position 6

<221> misc\_feature  
<222> (9)...(9)  
<223> y = t/u or c at position 9

<221> misc\_feature  
<222> (12)...(12)  
<223> b = g or c or t/u at position 12

<221> misc\_feature  
<222> (18)...(18)  
<223> r = g or a at position 18

<221> misc\_feature  
<222> (24)...(24)  
<223> b = g or c or t/u at position 24

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<221> misc_feature
<222> (30)...(30)
<223> y = t/u or c at position 30

<221> misc_feature
<222> (33)...(33)
<223> y = t/u or c at position 33

<221> misc_feature
<222> (36)...(36)
<223> b = g or c or t/u at position 36

<221> misc_feature
<222> (39)...(39)
<223> h = a or c or t/u at position 39

<221> misc_feature
<222> (42)...(42)
<223> h = a or c or t/u at position 42

<400> 1
gtbtaygayg tbaccgartg ggtbaagcgy cayccbghg gh 42

<210> 2
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
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<222> (3)...(3)
<223> h = a or c or t/u at position 3

<221> misc_feature
<222> (6)...(6)
<223> y = t/u or c at position 6

<221> misc_feature
<222> (12)...(12)
<223> y = t/u or c at position 12

<221> misc_feature
<222> (27)...(27)
<223> y = t/u or c at position 27

<221> misc_feature
<222> (33)...(33)
<223> y = t/u or c at position 33

<221> misc_feature
<222> (39)...(39)
<223> b = g or c or t/u at position 39

<221> misc_feature
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<222> (42)...(42)  
<223> y = t/u or c at position 42

<221> misc\_feature  
<222> (45)...(45)  
<223> y = t/u or c at position 45

<400> 2  
gghgcyclccg cyaactggtg gaagcaycag cayaacgtbc aycay 45

<210> 3  
<211> 45  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Reverse Primer RO836

<221> misc\_feature  
<222> (1)...(1)  
<223> r = g or a at position 1

<221> misc\_feature  
<222> (4)...(4)  
<223> r = g or a at position 4

<221> misc\_feature  
<222> (7)...(7)  
<223> v = a or g or c at position 7

<221> misc\_feature  
<222> (13)...(13)  
<223> r = g or a at position 13

<221> misc\_feature  
<222> (19)...(19)  
<223> r = g or a at position 19

<221> misc\_feature  
<222> (34)...(34)  
<223> r = g or a at position 34

<221> misc\_feature  
<222> (40)...(40)  
<223> r = g or a at position 40

<221> misc\_feature  
<222> (43)...(43)  
<223> d = a or g or t/u at position 43

<400> 3  
rtgrtgvacg ttrtgctgrt gcttccacca gttrgcggar gcdcc 45

<210> 4  
<211> 36  
<212> DNA  
<213> Artificial Sequence

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<220>
<223> Reverse Primer R0838

<221> misc_feature
<222> (6)...(6)
<223> r = g or a at position 6

<221> misc_feature
<222> (12)...(12)
<223> r = g or a at position 12

<221> misc_feature
<222> (15)...(15)
<223> y = t/u or c at position 15

<221> misc_feature
<222> (18)...(18)
<223> r = g or a at position 18

<221> misc_difference
<222> (21)...(21)
<223> r = g or a at position 21

<221> misc_feature
<222> (24)...(24)
<223> s = g or c at position 24

<221> misc_feature
<222> (27)...(27)
<223> r = g or a at position 27

<221> misc_feature
<222> (30)...(30)
<223> v = a or g or c at position 30

<400> 4
ttgatrgtct arctygtrgt rgasaarggv tggtag

<210> 5
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer R0753

<221> misc_feature
<222> (10)...(10)
<223> n = a or g or c or t/u, unknown or other at
position 10

<221> misc_feature
<222> (13)...(13)
<223> r = g or a at position 13

<221> misc_feature
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<222> (16)...(16)  
<223> n = a or g or c or t/u, unknown or other at position 16

<221> misc\_feature  
<222> (18)...(19)  
<223> r = g or a at positions 18-19

<221> misc\_feature  
<222> (22)...(22)  
<223> r = g or a at position 22

<400> 5  
catcatcatn ggraanarrt grtg

24

<210> 6  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer R0754

<221> misc\_feature  
<222> (15)...(15)  
<223> y = t/u or c at position 15

<221> misc\_feature  
<222> (18)...(18)  
<223> y = t/u or c at position 18

<221> misc\_feature  
<222> (21)...(21)  
<223> n = a or g or c or t/u, unknown, or other at position 21

<221> misc\_feature  
<222> (24)...(24)  
<223> y = t/u or c at position 24

<221> misc\_feature  
<222> (27)...(27)  
<223> n = a or g or c or t/u, unknown, or other at position 27

<221> misc\_feature  
<222> (30)...(30)  
<223> y = t/u or c at position 30

<400> 6  
ctactactac tacaycayac ntayacnaay

30

<210> 7  
<211> 31  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer R0898

<400> 7  
cccaagtcacg acgttgtaaa acgacggcca g 31

<210> 8  
<211> 31  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer R0930

<400> 8  
gacgattaac aaggtgattt cccaggatgt c 31

<210> 9  
<211> 38  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer R0973

<400> 9  
gactaactcg agtcacgtgg accaggccgt gaggtcct 38

<210> 10  
<211> 45  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer R0974

<400> 10  
gactaactcg agttgacgag gtttgtatgt tcggcggttt gcttg 45

<210> 11  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer R0899

<400> 11  
agcggataac aatttcacac agaaaacagc 30

<210> 12  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer R01004

<400> 12  
 tggctaccgt cgtgctggat gcaagttccg 30

<210> 13  
 <211> 39  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Primer R01046

<400> 13  
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<210> 14  
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<400> 14  
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 gacgacgcct ggtgcgcgat ccacggcacc gtgtacgaca tcaccaagtt cagcaaggtg 120  
 caccggcg gggacatcat catgctggcc gctggcaagg agggcaccat cctgttcgag 180  
 acgtaccaca tcaaggggcg cccggacgat gtgctgcgca agtacaaggt cggcaagctc 240  
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 tacaccaacc tcatcgagat ggagaacggt ttggccaagg tcaaggcgcc cgacgtcgac 720  
 ccgaagaagg tcgaccaggaa gagcgacccg gacgtcttca gtacgtaccc gatgctcg 780  
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<400> 15  
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 caccggcg gggacatcat catgctggcc gctggcaagg agggcaccat cctgttcgag 180

acgttaccaca	tcaaggggcgt	tccggacgcg	gtgctgcgc	agtacaagg	cgccaag	ctc	240
ccccaggggca	agaagggcga	aacgagccac	atgcccaccc	ggctcgactc	ggccttctac		300
tactcgtggg	acagcgagtt	ttacagggtg	ctccgcgagc	gcgtcgccaa	gaagctggcc		360
gagcccccgc	tcatgcagcg	cgcgcgcatg	gagctctggg	ccaaggcgat	cttcctctg		420
gcagggttct	ggggctccct	ttacgcccatt	tgcgtctag	acccgcacgg	cgggtccatg		480
gtagccgcg	ttacgctcg	cgtgttcgt	gcctttgtcg	gaacttgcatt	ccagcacgac		540
ggcagccacg	gcccattctc	caagtcgcga	ttcatgaaca	aggcggcggg	ctggaccctc		600
gacatgatcg	gcccgcagcgc	gatgacactgg	gagatgcagc	acgttctgg	ccaccacccg		660
tacaccaacc	tcatcgagat	ggagaacgg	ttggccaagg	tcaagggcgc	cgacgtcgac		720
ccgaagaagg	tcgaccagga	gagcgacccg	gacgtcttca	gtacgtaccc	gatgttgc		780
ctgcacccgt	ggcacgcga	gcccgtttac	cacaagttcc	agcacctgta	cgcggcg		840
atctttgggt	ttatgacgat	taacaagg	atttcccagg	atgtcggg	tgtgtcg		900
aagcgcctgt	tccagatcga	cgccaaactgc	cggttatggc	gcccctggta	cgtggcccgc		960
ttctggatca	tgaagctcct	caccacgctc	tacatgg	cgcttccat	gtacatgcag		1020
gggcctgctc	agggctgaa	gctttctt	atggcccact	tcacccgtgg	agaggcctc		1080
gccaccatgt	ttattgtcaa	ccacatcatc	gaggcgtca	gctacgctt	caaggacgcg		1140
gtcaaggcgc	tcatggctcc	gccgcgcact	gtgcacgg	tcaccccgat	gcagggtacg		1200
caaaaaggcgc	tcaagtgcggc	cgagtcgacc	aagtccgacg	ccgacaagac	gaccatgatc		1260
ccccctcaacg	actggggcgc	tgtgcagtgc	cagacccctg	tgaactggc	tgtcggtcg		1320
tggttttgg	accactttc	gggcggcctc	aaccaccaga	ttgagcacca	ctgcttcccc		1380
caaaaacccccc	acacggtcaa	cgtctacatc	tcagggatcg	tcaaggagac	ctgcgaagaa		1440
tacggcgtgc	cgtagccaggc	tgagatcagc	ctttctctg	cctattcaa	gatgtgtcg		1500
cacccctccgca	cgctcgccaa	cgaggacctc	acggcctggt	ccacgtga			1548

<210> 16  
<211> 1548

<212> DNA  
<213> *Thraustochytrium aureum*

<400> 16

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cacccggcg	gggacatcat	catgctggcc	gctggcaagg	aggccaccat	cctgttcgag	180
acctaccaca	tcaaggggcg	cccggacgcg	gtgctgcgca	agtacaagg	cggcaagctc	240
ccccaggggca	agaaggggca	aacgagccac	atgcccaccc	ggctcgactc	ggcctcctac	300
tactctgtgg	acagcgagt	ttacaggggt	ctccgcgagc	gcgtcgccaa	gaagctggcc	360
gagccggcc	tcatgcagcg	cgcgcgcatg	gagctctggg	ccaaggcgat	cttcctcctg	420
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ggcagccacg	ggcccttc	caagtgcgat	tcatgcata	aggccgggg	ctggaccctc	600
gacatgatcg	gcccggagcgc	gatgacccctgg	gagatgcagc	acgttcttgg	tcaccacccg	660
tacaccaacc	tcatcgagat	ggagaacgg	ttggcccaagg	tcaaggggcgc	cgacgtcgac	720
ccgaagaagg	tcgaccagga	gagcgcacccg	gacgtcttca	gtacgtaccc	gatgttgc	780
ctgcacccgt	ggcacggcca	gcggttttac	cacaagttcc	agcacctgta	cgcccccgtt	840
atctttgggt	ttatgacgat	taacaagg	atttcccagg	atgtcggggt	tgtgtcg	900
aagcgccctgt	tccagatcga	cgccaaactgc	cggttatggca	gcccctggaa	cggtggcccg	960
ttctggatca	tgaagctcct	caccacgctc	tacatgggtt	cgcttcccat	gtacatgcag	1020
gggcctgctc	agggttgaa	gctttcttc	atggcccact	tcacccgtgg	agaggtcctc	1080
gccaccatgt	ttattgtcaa	ccacatcatc	gagggcgtca	gctacgcttc	caaggacg	1140
gtcaaggcg	tcatggctcc	ggcgccact	gtgcacgg	tcaccccgat	gcaggtgac	1200
caaaaaggcgc	tcagtgcggc	cgagtgcacc	aagtgcgacg	ccgacaagac	gaccatgatc	1260
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tggttttgg	accacttttgc	gggcgccta	aaccaccaga	ttgagcacca	ctgcttcccc	1380
caaaaacccccc	acacggtcaa	cgtctacatc	tcgggcac	tcaaggagac	ctgcaagaa	1440
tacggcggtgc	cgtaccaggc	tgagatcagc	ctcttctctg	cctatttcaa	gatgtcg	1500
cacctccqca	cqctcqgca	cgaggac	acqccctqgt	ccacgtqa		1548

<210> 17  
 <211> 1548  
 <212> DNA  
 <213> Thraustochytrium aureum

<400> 17

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gacgacgcct	ggtgcgcgat	ccacggcacc	gtgtacgaca	tcaccaagtt	cagcaaggtg	120
cacccggcg	gggacatcat	catgctggcc	gctggcaagg	aggccacca	cctgttcgag	180
acctaccaca	tcaaggcg	cccgacgcg	gtgctgcga	agtacaaggt	cgccaagctc	240
ccccaggcga	agaaggcg	aacgagccac	atgcccaccc	ggctcgactc	ggcctctac	300
tactcgtgg	acagcgagtt	ttacagggtg	ctccgcgagc	gctgcgcctt	gaagctggcc	360
gagcccgcc	tcatgcagcg	cgcgcgcatt	gagctctgg	ccaaggcgat	cttcctcctt	420
gcaggttct	ggggctccct	ttacgccatg	tgcgtcttag	acccgcacgg	cggtgcacat	480
gtagccgccc	ttacgctcgg	cgtgttcgt	gcctttgtcg	gaacttgcac	ccagcacgac	540
ggcagccacg	gccccttc	caagtcgcga	ttcatgaaca	aggccggcgg	ctggaccctc	600
gacatgatcg	gcccgcgtgc	gtgacccatgg	gagatgcagc	acgttcttgg	ccaccaccccg	660
tacaccaacc	tcatcgagat	ggagaacgg	ttggccaaagg	tcaaggcg	cgacgtcgac	720
ccgaaaaagg	tgcaccagga	gaggcacc	gacgtttca	gtacgtaccc	gatgctcg	780
ctgcaccctgt	ggcaccgca	gcccgtttac	cacaagttcc	agcacctgt	cgcccccgtt	840
atcttgggt	tatgacat	taacaagggt	atttccagg	atgtcggggt	tgtgctgc	900
aagccctgt	tccagatca	cggcaactgc	cggtatggca	gcccctgtt	cggtggccgc	960
ttctggatca	tgaagctct	caccacgctc	tacatggtgg	cgcttccat	gtacatgcag	1020
gggcctgtc	agggcttggaa	gctttcttc	atggcccact	tcacctgcgg	agaggtcc	1080
gccaccatgt	ttattgtcaa	ccacatcatc	gagggcgtca	gctacgctt	caaggacgc	1140
gtcaaggcg	tcatggctcc	gcccgcact	gtgcacgg	tcaccccgat	gcaggtgac	1200
caaaaggcgc	tcagtgcggc	cgagtcggcc	aagtccggac	ccgacaagac	gaccatgatc	1260
cccctcaacg	actggccgc	tgtcagtgc	cagacctctg	tgaactggc	tgtcgggtcg	1320
tggtttgg	accactttc	ggccggcctc	aaccaccaga	ttgagcacca	ctgcttcccc	1380
caaaacccccc	acacggtaa	cgtctacatc	tcgggcacatc	tcaaggagac	ctgcgaagaa	1440
tacggcgtgc	cgtaccaggc	tgagatcagc	ctcttctctg	cctattcaa	gatgctgtcg	1500
caccccgca	cgctcgccaa	cgaggaccc	acggccttgt	ccacgtga		1548

<210> 18  
 <211> 515  
 <212> PRT  
 <213> Thraustochytrium aureum

<400> 18

Met	Thr	Val	Gly	Phe	Asp	Glu	Thr	Val	Thr	Met	Asp	Thr	Val	Arg	Asn
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His	Asn	Met	Pro	Asp	Asp	Ala	Trp	Cys	Ala	Ile	His	Gly	Thr	Val	Tyr
							20			25			30		
Asp	Ile	Thr	Lys	Phe	Ser	Lys	Val	His	Pro	Gly	Gly	Asp	Ile	Ile	Met
							35			40		45			
Leu	Ala	Ala	Gly	Lys	Glu	Ala	Thr	Ile	Leu	Phe	Glu	Thr	Tyr	His	Ile
							50			55		60			
Lys	Gly	Val	Pro	Asp	Ala	Val	Leu	Arg	Lys	Tyr	Lys	Val	Gly	Lys	Leu
							65			70		75		80	
Pro	Gln	Gly	Lys	Gly	Glu	Thr	Ser	His	Met	Pro	Thr	Gly	Leu	Asp	
							85			90		95			
Ser	Ala	Ser	Tyr	Tyr	Ser	Trp	Asp	Ser	Glu	Phe	Tyr	Arg	Val	Leu	Arg
							100			105		110			
Glu	Arg	Val	Ala	Lys	Lys	Leu	Ala	Glu	Pro	Gly	Leu	Met	Gln	Arg	Ala
							115			120		125			
Arg	Met	Glu	Leu	Trp	Ala	Lys	Ala	Ile	Phe	Leu	Leu	Ala	Gly	Phe	Trp
							130			135		140			

10/32

Gly Ser Leu Tyr Ala Met Cys Val Leu Asp Pro His Gly Gly Ala Met  
 145 150 155 160  
 Val Ala Ala Val Thr Leu Gly Val Phe Ala Ala Phe Val Gly Thr Cys  
 165 170 175  
 Ile Gln His Asp Gly Ser His Gly Ala Phe Ser Lys Ser Arg Phe Met  
 180 185 190  
 Asn Lys Ala Ala Gly Trp Thr Leu Asp Met Ile Gly Ala Ser Ala Met  
 195 200 205  
 Thr Trp Glu Met Gln His Val Leu Gly His His Pro Tyr Thr Asn Leu  
 210 215 220  
 Ile Glu Met Glu Asn Gly Leu Ala Lys Val Lys Gly Ala Asp Val Asp  
 225 230 235 240  
 Pro Lys Lys Val Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Thr Tyr  
 245 250 255  
 Pro Met Leu Arg Leu His Pro Trp His Arg Gln Arg Phe Tyr His Lys  
 260 265 270  
 Phe Gln His Leu Tyr Ala Pro Phe Ile Phe Gly Phe Met Thr Ile Asn  
 275 280 285  
 Lys Val Ile Ser Gln Asp Val Gly Val Val Leu Arg Lys Arg Leu Phe  
 290 295 300  
 Gln Ile Asp Ala Asn Cys Arg Tyr Gly Ser Pro Trp Tyr Val Ala Arg  
 305 310 315 320  
 Phe Trp Ile Met Lys Leu Leu Thr Thr Leu Tyr Thr Val Ala Leu Pro  
 325 330 335  
 Met Tyr Met Gln Gly Pro Ala Gln Gly Leu Lys Leu Phe Phe Met Ala  
 340 345 350  
 His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile Val Asn His  
 355 360 365  
 Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val  
 370 375 380  
 Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met Gln Val Thr  
 385 390 395 400  
 Gln Lys Ala Leu Ser Ala Ala Glu Ser Thr Lys Ser Asp Ala Asp Lys  
 405 410 415  
 Thr Thr Met Ile Pro Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr  
 420 425 430  
 Ser Val Asn Trp Ala Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly  
 435 440 445  
 Gly Leu Asn His Gln Ile Glu His His Cys Phe Pro Gln Asn Pro His  
 450 455 460  
 Thr Val Asn Val Tyr Ile Ser Gly Ile Val Lys Glu Thr Cys Glu Glu  
 465 470 475 480  
 Tyr Gly Val Pro Tyr Gln Ala Glu Ile Ser Leu Phe Ser Ala Tyr Phe  
 485 490 495  
 Lys Met Leu Ser His Leu Arg Thr Leu Gly Asn Glu Asp Leu Thr Ala  
 500 505 510  
 Arg Ser Thr  
 515

<210> 19

<211> 515

<212> PRT

<213> Thraustochytrium aureum

<400> 19

Met Thr Val

1 - 5 - 10 - 15

11/32

His Asn Met Pro Asp Asp Ala Trp Cys Ala Ile His Gly Thr Val Tyr  
 20 25 30  
 Asp Ile Thr Lys Phe Ser Lys Val His Pro Gly Gly Asp Ile Ile Met  
 35 40 45  
 Leu Ala Ala Gly Lys Glu Ala Thr Ile Leu Phe Glu Thr Tyr His Ile  
 50 55 60  
 Lys Gly Val Pro Asp Ala Val Leu Arg Lys Tyr Lys Val Gly Lys Leu  
 65 70 75 80  
 Pro Gln Gly Lys Lys Gly Glu Thr Ser His Met Pro Thr Gly Leu Asp  
 85 90 95  
 Ser Ala Phe Tyr Tyr Ser Trp Asp Ser Glu Phe Tyr Arg Val Leu Arg  
 100 105 110  
 Glu Arg Val Ala Lys Lys Leu Ala Glu Pro Gly Leu Met Gln Arg Ala  
 115 120 125  
 Arg Met Glu Leu Trp Ala Lys Ile Phe Leu Leu Ala Gly Phe Trp  
 130 135 140  
 Gly Ser Leu Tyr Ala Met Cys Val Leu Asp Pro His Gly Gly Ala Met  
 145 150 155 160  
 Val Ala Ala Val Thr Leu Gly Val Phe Ala Ala Phe Val Gly Thr Cys  
 165 170 175  
 Ile Gln His Asp Gly Ser His Gly Ala Phe Ser Lys Ser Arg Phe Met  
 180 185 190  
 Asn Lys Ala Ala Gly Trp Thr Leu Asp Met Ile Gly Ala Ser Ala Met  
 195 200 205  
 Thr Trp Glu Met Gln His Val Leu Gly His His Pro Tyr Thr Asn Leu  
 210 215 220  
 Ile Glu Met Glu Asn Gly Leu Ala Lys Val Lys Gly Ala Asp Val Asp  
 225 230 235 240  
 Pro Lys Lys Val Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Thr Tyr  
 245 250 255  
 Pro Met Leu Arg Leu His Pro Trp His Arg Gln Arg Phe Tyr His Lys  
 260 265 270  
 Phe Gln His Leu Tyr Ala Pro Phe Ile Phe Gly Phe Met Thr Ile Asn  
 275 280 285  
 Lys Val Ile Ser Gln Asp Val Gly Val Val Leu Arg Lys Arg Leu Phe  
 290 295 300  
 Gln Ile Asp Ala Asn Cys Arg Tyr Gly Ser Pro Trp Tyr Val Ala Arg  
 305 310 315 320  
 Phe Trp Ile Met Lys Leu Leu Thr Thr Leu Tyr Met Val Ala Leu Pro  
 325 330 335  
 Met Tyr Met Gln Gly Pro Ala Gln Gly Leu Lys Leu Phe Phe Met Ala  
 340 345 350  
 His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile Val Asn His  
 355 360 365  
 Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val  
 370 375 380  
 Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met Gln Val Thr  
 385 390 395 400  
 Gln Lys Ala Leu Ser Ala Ala Glu Ser Thr Lys Ser Asp Ala Asp Lys  
 405 410 415  
 Thr Thr Met Ile Pro Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr  
 420 425 430  
 Ser Val Asn Trp Ala Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly  
 435 440 445  
 Gly Leu Asn His Gln Ile Glu His His Cys Phe Pro Gln Asn Pro His  
 450 455 460  
 Thr Val Asn Val Tyr Ile Ser Gly Ile Val Lys Glu Thr Cys Glu Glu

12/32

465	470	475	480												
Tyr	Gly	Val	Pro	Tyr	Gln	Ala	Glu	Ile	Ser	Leu	Phe	Ser	Ala	Tyr	Phe
					485					490					495
Lys	Met	Leu	Ser	His	Leu	Arg	Thr	Leu	Gly	Asn	Glu	Asp	Leu	Thr	Ala
					500				505						510
Trp	Ser	Thr													
					515										

&lt;210&gt; 20

&lt;211&gt; 515

&lt;212&gt; PRT

&lt;213&gt; Thraustochytrium aureum

&lt;400&gt; 20

Met	Thr	Val	Gly	Phe	Asp	Glu	Thr	Val	Thr	Met	Asp	Thr	Val	Arg	Asn
1					5					10					15
His	Asn	Met	Pro	Asp	Asp	Ala	Trp	Cys	Ala	Ile	His	Gly	Thr	Val	Tyr
										20		25			30
Asp	Ile	Thr	Lys	Phe	Ser	Lys	Val	His	Pro	Gly	Gly	Asp	Ile	Ile	Met
										35		40			45
Leu	Ala	Ala	Gly	Lys	Glu	Ala	Thr	Ile	Leu	Phe	Glu	Thr	Tyr	His	Ile
										50		55			60
Lys	Gly	Val	Pro	Asp	Ala	Val	Leu	Arg	Lys	Tyr	Lys	Val	Gly	Lys	Leu
										65		70			80
Pro	Gln	Gly	Lys	Lys	Gly	Glu	Thr	Ser	His	Met	Pro	Thr	Gly	Leu	Asp
										85		90			95
Ser	Ala	Ser	Tyr	Tyr	Ser	Trp	Asp	Ser	Glu	Phe	Tyr	Arg	Val	Leu	Arg
										100		105			110
Glu	Arg	Val	Ala	Lys	Lys	Leu	Ala	Glu	Pro	Gly	Leu	Met	Gln	Arg	Ala
										115		120			125
Arg	Met	Glu	Leu	Trp	Ala	Lys	Ala	Ile	Phe	Leu	Leu	Ala	Gly	Phe	Trp
										130		135			140
Gly	Ser	Leu	Tyr	Ala	Met	Cys	Val	Leu	Asp	Pro	His	Gly	Gly	Ala	Met
										145		150			160
Val	Ala	Ala	Val	Thr	Leu	Gly	Val	Phe	Ala	Ala	Phe	Val	Gly	Thr	Cys
										165		170			175
Ile	Gln	His	Asp	Gly	Ser	His	Gly	Ala	Phe	Ser	Lys	Ser	Arg	Phe	Met
										180		185			190
Asn	Lys	Ala	Ala	Gly	Trp	Thr	Leu	Asp	Met	Ile	Gly	Ala	Ser	Ala	Met
										195		200			205
Thr	Trp	Glu	Met	Gln	His	Val	Leu	Gly	His	His	Pro	Tyr	Thr	Asn	Leu
										210		215			220
Ile	Glu	Met	Glu	Asn	Gly	Leu	Ala	Lys	Val	Lys	Gly	Ala	Asp	Val	Asp
										225		230			240
Pro	Lys	Lys	Val	Asp	Gln	Glu	Ser	Asp	Pro	Asp	Val	Phe	Ser	Thr	Tyr
										245		250			255
Pro	Met	Leu	Arg	Leu	His	Pro	Trp	His	Arg	Gln	Arg	Phe	Tyr	His	Lys
										260		265			270
Phe	Gln	His	Leu	Tyr	Ala	Pro	Leu	Ile	Phe	Gly	Phe	Met	Thr	Ile	Asn
										275		280			285
Lys	Val	Ile	Ser	Gln	Asp	Val	Gly	Val	Val	Leu	Arg	Lys	Arg	Leu	Phe
										290		295			300
Gln	Ile	Asp	Ala	Asn	Cys	Arg	Tyr	Gly	Ser	Pro	Trp	Asn	Val	Ala	Arg
										305		310			320
Phe	Trp	Ile	Met	Lys	Leu	Leu	Thr	Leu	Tyr	Met	Val	Ala	Leu	Pro	
										325		330			335
Met	Tyr	Met	Gln	Gly	Pro	Ala	Gln	Gly	Leu	Lys	Leu	Phe	Phe	Met	Ala

340	345	350
His Phe Thr Cys Gly Glu Val	Leu Ala Thr Met Phe Ile Val Asn His	
355	360	365
Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val		
370	375	380
Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met Gln Val Thr		
385	390	395
Gln Lys Ala Leu Ser Ala Ala Glu Ser Thr Lys Ser Asp Ala Asp Lys		
405	410	415
Thr Thr Met Ile Pro Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr		
420	425	430
Ser Val Asn Trp Ala Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly		
435	440	445
Gly Leu Asn His Gln Ile Glu His His Cys Phe Pro Gln Asn Pro His		
450	455	460
Thr Val Asn Val Tyr Ile Ser Gly Ile Val Lys Glu Thr Cys Glu Glu		
465	470	475
Tyr Gly Val Pro Tyr Gln Ala Glu Ile Ser Leu Phe Ser Ala Tyr Phe		
485	490	495
Lys Met Leu Ser His Leu Arg Thr Leu Gly Asn Glu Asp Leu Thr Ala		
500	505	510
Trp Ser Thr		
515		

&lt;210&gt; 21

&lt;211&gt; 515

&lt;212&gt; PRT

&lt;213&gt; Thraustochytrium aureum

&lt;400&gt; 21

Met Thr Val Gly Phe Asp Glu Thr Val Thr Met Asp Thr Val Arg Asn			
1	5	10	15
His Asn Met Pro Asp Asp Ala Trp Cys Ala Ile His Gly Thr Val Tyr			
20	25	30	
Asp Ile Thr Lys Phe Ser Lys Val His Pro Gly Gly Asp Ile Ile Met			
35	40	45	
Leu Ala Ala Gly Lys Glu Ala Thr Ile Leu Phe Glu Thr Tyr His Ile			
50	55	60	
Lys Gly Val Pro Asp Ala Val Leu Arg Lys Tyr Lys Val Gly Lys Leu			
65	70	75	80
Pro Gln Gly Lys Gly Glu Thr Ser His Met Pro Thr Gly Leu Asp			
85	90	95	
Ser Ala Ser Tyr Tyr Ser Trp Asp Ser Glu Phe Tyr Arg Val Leu Arg			
100	105	110	
Glu Arg Val Ala Lys Lys Leu Ala Glu Pro Gly Leu Met Gln Arg Ala			
115	120	125	
Arg Met Glu Leu Trp Ala Lys Ala Ile Phe Leu Leu Ala Gly Phe Trp			
130	135	140	
Gly Ser Leu Tyr Ala Met Cys Val Leu Asp Pro His Gly Gly Ala Met			
145	150	155	160
Val Ala Ala Val Thr Leu Gly Val Phe Ala Ala Phe Val Gly Thr Cys			
165	170	175	
Ile Gln His Asp Gly Ser His Gly Ala Phe Ser Lys Ser Arg Phe Met			
180	185	190	
Asn Lys Ala Ala Gly Trp Thr Leu Asp Met Ile Gly Ala Ser Ala Met			
195	200	205	
Thr Trp Glu Met Gln His Val Leu Gly His His Pro Tyr Thr Asn Leu			

210	215	220														
Ile	Glu	Met	Glu	Asn	Gly	Leu	Ala	Lys	Val	Lys	Gly	Ala	Asp	Val	Asp	
225			230			235										240
Pro	Lys	Lys	Val	Asp	Gln	Glu	Ser	Asp	Pro	Asp	Val	Phe	Ser	Thr	Tyr	
	245						250								255	
Pro	Met	Leu	Arg	Leu	His	Pro	Trp	His	Arg	Gln	Arg	Phe	Tyr	His	Lys	
	260						265								270	
Phe	Gln	His	Leu	Tyr	Ala	Pro	Phe	Ile	Phe	Gly	Ser	Met	Thr	Ile	Asn	
	275						280								285	
Lys	Val	Ile	Ser	Gln	Asp	Val	Gly	Val	Val	Leu	Arg	Lys	Arg	Leu	Phe	
	290						295								300	
Gln	Ile	Asp	Ala	Asn	Cys	Arg	Tyr	Gly	Ser	Pro	Trp	Tyr	Val	Ala	Arg	
	305						310								320	
Phe	Trp	Ile	Met	Lys	Leu	Leu	Thr	Thr	Leu	Tyr	Met	Val	Ala	Leu	Pro	
							325								335	
Met	Tyr	Met	Gln	Gly	Pro	Ala	Gln	Gly	Leu	Lys	Leu	Phe	Phe	Met	Ala	
							340								350	
His	Phe	Thr	Cys	Gly	Glu	Val	Leu	Ala	Thr	Met	Phe	Ile	Val	Asn	His	
							355								365	
Ile	Ile	Glu	Gly	Val	Ser	Tyr	Ala	Ser	Lys	Asp	Ala	Val	Lys	Gly	Val	
	370						375								380	
Met	Ala	Pro	Pro	Arg	Thr	Val	His	Gly	Val	Thr	Pro	Met	Gln	Val	Thr	
	385						390								400	
Gln	Lys	Ala	Leu	Ser	Ala	Ala	Glu	Ser	Ala	Lys	Ser	Asp	Ala	Asp	Lys	
							405								415	
Thr	Thr	Met	Ile	Pro	Leu	Asn	Asp	Trp	Ala	Ala	Val	Gln	Cys	Gln	Thr	
							420								430	
Ser	Val	Asn	Trp	Ala	Val	Gly	Ser	Trp	Phe	Trp	Asn	His	Phe	Ser	Gly	
							435								445	
Gly	Leu	Asn	His	Gln	Ile	Glu	His	His	Cys	Phe	Pro	Gln	Asn	Pro	His	
							450								460	
Thr	Val	Asn	Val	Tyr	Ile	Ser	Gly	Ile	Val	Lys	Glu	Thr	Cys	Glu	Glu	
	465						470								480	
Tyr	Gly	Val	Pro	Tyr	Gln	Ala	Glu	Ile	Ser	Leu	Phe	Ser	Ala	Tyr	Phe	
							485								495	
Lys	Met	Leu	Ser	His	Leu	Arg	Thr	Leu	Gly	Asn	Glu	Asp	Leu	Thr	Ala	
							500								510	
Trp	Ser	Thr														
		515														

&lt;210&gt; 22

&lt;211&gt; 879

&lt;212&gt; DNA

&lt;213&gt; Mus musculus

&lt;400&gt; 22

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ccacagatt	ctcgagtcg	cgggtggttc	ctgctggact	cttaccttcc	caccttcatc	120
ctcacccatca	cgtacctgt	ctcgatatgg	ctgggttaaca	agtacatgaa	gaacaggcct	180
gctctgtctc	tcagggccat	cctcaccttg	tataacctcg	caatcacact	tctttctgct	240
tatatgctgg	tggagctcat	cctctccagc	tgggaaggag	gttacaactt	gcagtgtcag	300
aatctcgaca	gtgcaggaga	aggtgatgtc	cgggttagcca	aggtcttgc	gtggtaactac	360
ttctccaaac	tagtggagtt	cctggacacg	attttcttgc	ttctacgaaa	aaagaccaat	420
cagatcacct	tccttcatgt	ctatcaccac	gcgtccatgt	tcaacatctg	gtgggtgttt	480
ttgaactgga	taccttggtt	tcaaagcttc	tttggaccca	ccctgaacag	ctttatccac	540
attctcatgt	actcctacta	cggctgtct	gtgttcccgt	ccatgcacaa	gtacctttgg	600
tggaagaagt	acctcacaca	ggctcagtcg	gtgcagttcg	tactcaccat	cacgcacacg	660

ctgagtgccg tggtaagcc ctgtggctc cccttggct gtctcatctt ccagtcttcc	720
tatatgatga cgctggtcat cctgttctta aacttctata ttcaagacata ccggaaaaag	780
ccagtgaaga aagagctgca agagaaagaa gtgaagaatg gttcccaa agcccactta	840
attgtggcta atggcatgac ggacaagaag gctcaataa	879

&lt;210&gt; 23

&lt;211&gt; 292

&lt;212&gt; PRT

<213> *Mus musculus*

&lt;400&gt; 23

Met Glu Gln Leu Lys Ala Phe Asp Asn Glu Val Asn Ala Phe Leu Asp	
1 5 10 15	
Asn Met Phe Gly Pro Arg Asp Ser Arg Val Arg Gly Trp Phe Leu Leu	
20 25 30	
Asp Ser Tyr Leu Pro Thr Phe Ile Leu Thr Ile Thr Tyr Leu Leu Ser	
35 40 45	
Ile Trp Leu Gly Asn Lys Tyr Met Lys Asn Arg Pro Ala Leu Ser Leu	
50 55 60	
Arg Gly Ile Leu Thr Leu Tyr Asn Leu Ala Ile Thr Leu Leu Ser Ala	
65 70 75 80	
Tyr Met Leu Val Glu Leu Ile Leu Ser Ser Trp Glu Gly Gly Tyr Asn	
85 90 95	
Leu Gln Cys Gln Asn Leu Asp Ser Ala Gly Glu Gly Asp Val Arg Val	
100 105 110	
Ala Lys Val Leu Trp Trp Tyr Phe Ser Lys Leu Val Glu Phe Leu	
115 120 125	
Asp Thr Ile Phe Phe Val Leu Arg Lys Lys Thr Asn Gln Ile Thr Phe	
130 135 140	
Leu His Val Tyr His His Ala Ser Met Phe Asn Ile Trp Trp Cys Val	
145 150 155 160	
Leu Asn Trp Ile Pro Cys Gly Gln Ser Phe Phe Gly Pro Thr Leu Asn	
165 170 175	
Ser Phe Ile His Ile Leu Met Tyr Ser Tyr Tyr Gly Leu Ser Val Phe	
180 185 190	
Pro Ser Met His Lys Tyr Leu Trp Trp Lys Lys Tyr Leu Thr Gln Ala	
195 200 205	
Gln Leu Val Gln Phe Val Leu Thr Ile Thr His Thr Leu Ser Ala Val	
210 215 220	
Val Lys Pro Cys Gly Phe Pro Phe Gly Cys Leu Ile Phe Gln Ser Ser	
225 230 235 240	
Tyr Met Met Thr Leu Val Ile Leu Phe Leu Asn Phe Tyr Ile Gln Thr	
245 250 255	
Tyr Arg Lys Lys Pro Val Lys Lys Glu Leu Gln Glu Lys Glu Val Lys	
260 265 270	
Asn Gly Phe Pro Lys Ala His Leu Ile Val Ala Asn Gly Met Thr Asp	
275 280 285	
Lys Lys Ala Gln	
290	

&lt;210&gt; 24

&lt;211&gt; 754

&lt;212&gt; DNA

<213> *Schizophytrium aggregatum*

&lt;400&gt; 24

ccagtgtgct ggaattcagg tactactact acaccatact tacacgaacc tgatcgagat

60

ggagaacggc	acccaaaagg	tcacccacgc	cgacgtcgac	cccaagaagg	ccgaccagga	120
gagcgacccg	gacgtcttca	gcaccttaccc	catgtccgt	ctgcacccgt	ggcaccgcaa	180
gcgcttctac	caccgcttcc	agcacctgt	cgcgccgctg	ctcttcgggtt	tcatgaccat	240
caacaaggtg	atcacccagg	atgtgggagt	tgtcctcagc	aagcgtctgt	ttcagatcga	300
tgccaaactgc	cgttacgcca	gcaagtgcgt	cgttgcgcgc	ttctggatca	tgaagctgct	360
caccgttctc	tacatggtcg	ccctccccgt	gtacacccag	ggccttgcgt	acgggctcaa	420
gcttttcttc	atcgcccaact	tttctgtgcgg	cgagctgctg	gccaccatgt	tcatcgtaa	480
ccacatcatc	gagggcgtct	cgtacgcctc	caaggactct	gtcaaggggca	ccatggcc	540
gccgcgcacg	gtgcacggcg	tgaccccgat	gcatgacacc	cgcgacgcgc	tcggcaagga	600
gaaggcagcc	accaagcacg	tgcgcgtcaa	cgactggcc	gccccgt	gccagaccc	660
ggtcaactgg	tcgatcgct	cgtggttctg	gaaccacttc	tccggcgggc	tcaaccacca	720
gatcgagcac	cacccccc	ccatgtatgt	gatg			754

&lt;210&gt; 25

&lt;211&gt; 251

&lt;212&gt; PRT

&lt;213&gt; Schizochytrium aggregatum

&lt;400&gt; 25

Gln	Cys	Ala	Gly	Ile	Gln	Val	Leu	Leu	Leu	His	His	Thr	Tyr	Thr	Asn
1					5				10					15	
Leu	Ile	Glu	Met	Glu	Asn	Gly	Thr	Gln	Lys	Val	Thr	His	Ala	Asp	Val
							20		25					30	
Asp	Pro	Lys	Lys	Ala	Asp	Gln	Glu	Ser	Asp	Pro	Asp	Val	Phe	Ser	Thr
							35		40				45		
Tyr	Pro	Met	Leu	Arg	Leu	His	Pro	Trp	His	Arg	Lys	Arg	Phe	Tyr	His
							50		55				60		
Arg	Phe	Gln	His	Leu	Tyr	Ala	Pro	Leu	Leu	Phe	Gly	Phe	Met	Thr	Ile
							65		70			75		80	
Asn	Lys	Val	Ile	Thr	Gln	Asp	Val	Gly	Val	Val	Leu	Ser	Lys	Arg	Leu
							85				90		95		
Phe	Gln	Ile	Asp	Ala	Asn	Cys	Arg	Tyr	Ala	Ser	Lys	Ser	Tyr	Val	Ala
							100			105			110		
Arg	Phe	Trp	Ile	Met	Lys	Leu	Leu	Thr	Val	Leu	Tyr	Met	Val	Ala	Leu
							115		120				125		
Pro	Val	Tyr	Thr	Gln	Gly	Leu	Val	Asp	Gly	Leu	Lys	Leu	Phe	Phe	Ile
							130		135				140		
Ala	His	Phe	Ser	Cys	Gly	Glu	Leu	Leu	Ala	Thr	Met	Phe	Ile	Val	Asn
							145		150				155		160
His	Ile	Ile	Glu	Gly	Val	Ser	Tyr	Ala	Ser	Lys	Asp	Ser	Val	Lys	Gly
							165			170			175		
Thr	Met	Ala	Pro	Pro	Arg	Thr	Val	His	Gly	Val	Thr	Pro	Met	His	Asp
							180			185			190		
Thr	Arg	Asp	Ala	Leu	Gly	Lys	Glu	Lys	Ala	Ala	Thr	Lys	His	Val	Pro
							195			200			205		
Leu	Asn	Asp	Trp	Ala	Ala	Val	Gln	Cys	Gln	Thr	Ser	Val	Asn	Trp	Ser
							210		215				220		
Ile	Gly	Ser	Trp	Phe	Trp	Asn	His	Phe	Ser	Gly	Gly	Leu	Asn	His	Gln
							225		230			235		240	
Ile	Glu	His	His	Leu	Phe	Pro	Met	Met	Met						
							245			250					

&lt;210&gt; 26

&lt;211&gt; 27

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

<220>  
<223> Primer RO1240

<400> 26  
ccctcgatga tgtggttgac gatgaac 27

<210> 27  
<211> 26  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> 5' Nested Primer RO1239

<400> 27  
cggagcatgg ggttaggtgct gaagac 26

<210> 28  
<211> 24  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer RO1236

<400> 28  
ccaactgccc ttacgccc aagt 24

<210> 29  
<211> 28  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> 3' Nested Primer RO1237

<400> 29  
caagctcttc ttcatcgccc acttttcg 28

<210> 30  
<211> 23  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer RO1240 and GeneRacer 5' Primer

<400> 30  
cgactggagc acgaggacac tga 23

<210> 31  
<211> 25  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer RO1236 and GeneRacer 3' Primer

<400> 31  
gctgtcaacg atacgctacg taacg 25

<210> 32  
<211> 26  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Nested Primer RO1239 and GeneRacer Nested 5' Primer

<400> 32  
ggacactgac atggactgaa ggagta 26

<210> 33  
<211> 23  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Nested Primer RO1237 and GeneRacer Nested 3' Primer

<400> 33  
cgctacgtaa cggcatgaca gtg 23

<210> 34  
<211> 34  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer RO1241

<400> 34  
gatatcgaat tcatgacggt gggcgccgat gagg 34

<210> 35  
<211> 34  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer RO1242

<400> 35  
gtacttaagc tttcacttgg acttgggtg gtcc 34

<210> 36  
<211> 1530  
<212> DNA  
<213> Schizophytrium aggregatum

<400> 36  
atgacgggtgg gcggcgatga ggtgtacagc atggcgcagg tgcgcgacca caacaccccg 60

gacgacgcct	ggtgtcgccat	ccacggcgag	gtgtacgagc	tgacccaagtt	cgtttcgacc	120
caccccgggg	gggacatcat	cttgcgtggc	gcccggcaagg	aggccaccat	cctgttcgag	180
acgttaccacg	tgcgccccat	ctccgacgcg	gtcctgcgca	agtaaccgcat	cggcaagctc	240
gccggcccg	gcaaggatga	gcccggcaac	gacagcacct	actacagctg	ggacagcgac	300
ttttacaagg	tgctccgcca	gctgtcggt	gcccgcctcg	aggagcgcaa	gatcgcccgc	360
cgcggcgcc	ccgagatctg	gatcaaggcc	gccatcctcg	tcaagcggctt	ctggtccatg	420
ctctacatca	tgtgcaccc	ggacccgaaac	cgccggcgcca	tcctggccgc	catcgcgctg	480
ggcgcgtcg	ccgccttcgt	cggcacgtgc	attcagcacg	acggcaacca	cggcgcgttc	540
gccttctctc	cggtcatgaa	caagctctct	ggctggacgc	tgcacatgat	cggcgcctagt	600
gccatgacct	gggagatgca	gcacgtgctg	ggccaccacc	cgtacaccaa	cctgatcgag	660
atggagaacg	gcacccaaa	ggtgcacccac	gcccgcgtcg	accccaagaa	ggccgaccag	720
gagagcggacc	cggtacgttt	cagcacctac	cccatgtcc	gtctgcaccc	gtggcaccgc	780
aagcgcttct	accaccgctt	ccagcacctg	tacgcgcgc	tgtctttcgg	tttcatgacc	840
atcaacaagg	tgatcaccca	ggatgtggga	gttgcctca	gcaagcgtct	gtttcagatc	900
gatgcaact	gcccgtacgc	cagcaagtgc	tacgttgcgc	gttcttgat	catgaagctg	960
ctcaccgtcc	tctacatggt	cgccctcccc	gtgtacaccc	aggcccttgc	cgacggcgtc	1020
aagctttct	tcatcgccca	ctttcgtgc	ggcgagctgc	tggccaccat	gttcatcgtc	1080
aaccacatca	tcgagggcgt	ctcgtaaccc	tccaaaggact	ctgtcaaggg	caccatggcg	1140
ccgccccgca	cggtgcacgg	cgtgaccccg	atgcatgaca	cccgcgacgc	gctcggaag	1200
gagaaggcag	ccaccaagca	cgtgcccgtc	aacgacttggg	ccggcggtcca	gtgccagacc	1260
tcggtaact	ggtcgatcgg	ctcggttgc	tggaaaccact	tctccggcgg	gctcaaccac	1320
cagatcgagc	accaccttct	ccccggcctc	acccacacca	cctacgtgta	cattcaggat	1380
gtgggtcagg	cgacgtgcgc	cgagtacggg	gtcccgtacc	agtcggagca	gagcctcttc	1440
tccgcctact	tcaagatgct	ctcccaccc	cgggcgctcg	gcaacgagcc	gatccctcg	1500
tgggagaagg	accaccccaa	gtccaaagtga				1530

&lt;210&gt; 37

&lt;211&gt; 509

&lt;212&gt; PRT

&lt;213&gt; Schizochytrium aggregatum

&lt;400&gt; 37

Met	Thr	Val	Gly	Gly	Asp	Glu	Val	Tyr	Ser	Met	Ala	Gln	Val	Arg	Asp
1			5			10				15					
His	Asn	Thr	Pro	Asp	Asp	Ala	Trp	Cys	Ala	Ile	His	Gly	Glu	Val	Tyr
			20				25				30				
Glu	Leu	Thr	Lys	Phe	Ala	Arg	Thr	His	Pro	Gly	Gly	Asp	Ile	Ile	Leu
			35				40			45					
Leu	Ala	Ala	Gly	Lys	Glu	Ala	Thr	Ile	Leu	Phe	Glu	Thr	Tyr	His	Val
			50			55			60						
Arg	Pro	Ile	Ser	Asp	Ala	Val	Leu	Arg	Lys	Tyr	Arg	Ile	Gly	Lys	Leu
			65			70			75			80			
Ala	Ala	Ala	Gly	Lys	Asp	Glu	Pro	Ala	Asn	Asp	Ser	Thr	Tyr	Tyr	Ser
			85			90			95						
Trp	Asp	Ser	Asp	Phe	Tyr	Lys	Val	Leu	Arg	Gln	Arg	Val	Val	Ala	Arg
			100			105			110						
Leu	Glu	Glu	Arg	Lys	Ile	Ala	Arg	Arg	Gly	Gly	Pro	Glu	Ile	Trp	Ile
			115			120			125						
Lys	Ala	Ala	Ile	Leu	Val	Ser	Gly	Phe	Trp	Ser	Met	Leu	Tyr	Leu	Met
			130			135			140						
Cys	Thr	Leu	Asp	Pro	Asn	Arg	Gly	Ala	Ile	Leu	Ala	Ala	Ile	Ala	Leu
			145			150			155			160			
Gly	Ile	Val	Ala	Ala	Phe	Val	Gly	Thr	Cys	Ile	Gln	His	Asp	Gly	Asn
			165			170			175						
His	Gly	Ala	Phe	Ala	Phe	Ser	Pro	Phe	Met	Asn	Lys	Leu	Ser	Gly	Trp
			180			185			190						
Thr	Leu	Asp	Met	Ile	Gly	Ala	Ser	Ala	Met	Thr	Trp	Glu	Met	Gln	His

20/32

195	200	205
Val	Leu	Gly
His	His	His
Pro	Tyr	Thr
Asn	Leu	Ile
Glu		
Met		
Glu		
Asn		
Gly		
210	215	220
Thr	Gln	Lys
Val	Thr	His
His	Ala	Asp
Val	Asp	Pro
Lys		Lys
Ala		Ala
Asp		Asp
Gln		Gln
225	230	235
Glu	Ser	Asp
Asp	Pro	Val
Pro	Asp	Phe
Asp	Val	Ser
Val	Phe	Thr
Phe	Ser	Tyr
245	250	255
Pro	Trp	His
His	Arg	Lys
Arg	Phe	Tyr
Phe	Tyr	His
Tyr	Arg	Arg
260	265	270
Pro	Leu	Leu
Leu	Phe	Gly
Phe	Met	Thr
Met	Ile	Asn
Ile	Asn	Lys
Asp	Val	Val
275	280	285
Val	Gly	Val
Val	Leu	Ser
Leu	Ser	Lys
Ser	Arg	Leu
Arg	Phe	Gln
Phe	Gln	Ile
Gln	Ile	Asp
Asp	Ala	Asn
Ala	Cys	
290	295	300
Arg	Tyr	Ala
Ala	Ser	Lys
Ser	Tyr	Val
Val	Ala	Arg
Ala	Phe	Tyr
Phe	Tyr	Trp
Tyr	Ile	Met
Met	Lys	Lys
Lys	Leu	
305	310	315
Leu	Thr	Val
Thr	Leu	Tyr
Leu	Tyr	Met
Tyr	Met	Val
Met	Ala	Leu
Ala	Leu	Pro
Leu	Pro	Val
Pro	Tyr	Thr
Tyr	Thr	Gln
325	330	335
Val	Asp	Gly
Gly	Leu	Lys
Leu	Lys	Leu
Phe	Phe	Ile
Ile	Ala	His
His	Phe	Ser
Phe	Ser	Cys
Ser	Cys	Gly
Gly	Glu	Glu
340	345	350
Leu	Leu	Ala
Ala	Thr	Met
Thr	Phe	Ile
Ile	Val	Asn
Asn	Ile	Ile
Ile	Glu	Gly
Gly	Gly	Val
355	360	365
Tyr	Ala	Ser
Ala	Ser	Lys
Ser	Asp	Ser
Asp	Val	Lys
Val	Lys	Gly
Gly	Thr	Thr
Thr	Met	Asp
Asp	Asp	Thr
Thr	Arg	Asp
Asp	Ala	Leu
Leu	Gly	Lys
370	375	380
Val	His	Gly
Gly	Val	Thr
Thr	Pro	Met
Pro	His	Asp
His	Arg	Thr
Arg	Asp	Asp
Asp	Ala	Leu
Leu	Gly	Lys
385	390	395
Glu	Lys	Ala
Ala	Ala	Thr
Thr	Lys	His
His	Val	Val
Val	Pro	Leu
Pro	Asn	Asp
Asn	Trp	Ala
Trp	Ala	Ala
Ala	Val	
405	410	415
Gln	Cys	Gln
Gln	Thr	Ser
Thr	Ser	Val
Val	Asn	Trp
Asn	Ile	Gly
Ile	Ser	Trp
Ser	Trp	Phe
Phe	Trp	Asn
420	425	430
His	Phe	Ser
Ser	Gly	Gly
Gly	Leu	Leu
Leu	Asn	His
Asn	Gln	Ile
Ile	Glu	His
His	His	Leu
Leu	Phe	Pro
435	440	445
Gly	Leu	Thr
Leu	Thr	His
Thr	Thr	Tyr
Tyr	Val	Tyr
Val	Tyr	Ile
Ile	Gln	Asp
Gln	Asp	Val
Asp	Val	Val
Val	Gln	Ala
450	455	460
Thr	Cys	Ala
Ala	Glu	Tyr
Tyr	Gly	Val
Val	Pro	Tyr
Pro	Gln	Ser
Gln	Ser	Glu
Ser	Gln	Ser
Gln	Ser	Leu
Leu	Phe	
465	470	475
Ser	Ala	Tyr
Ala	Tyr	Phe
Phe	Lys	Met
Met	Leu	Ser
Leu	Ser	His
His	Leu	Arg
Leu	Arg	Ala
Arg	Ala	Leu
Ala	Gly	Gly
Gly	Asn	Asn
Asn	Glu	
485	490	495
Pro	Met	Pro
Pro	Ser	Trp
Trp	Glu	Lys
Lys	Asp	His
Asp	His	Pro
Pro	Lys	Ser
Lys		
500	505	

&lt;210&gt; 38

&lt;211&gt; 32

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Primer R01201

&lt;400&gt; 38

cgtgttcgct gcctttgtcg gaacttgcatt cc

32

&lt;210&gt; 39

&lt;211&gt; 33

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

21/32

&lt;223&gt; Primer RO1202

&lt;400&gt; 39

ttgacaataa acatggaggc gaggacctct ccg

33

&lt;210&gt; 40

&lt;211&gt; 30

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Primer RO1210

&lt;400&gt; 40

gctggttgga ctttggacat gattggatcc

30

&lt;210&gt; 41

&lt;211&gt; 30

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Primer RO1211

&lt;400&gt; 41

tacattggca ggccaaccat gtagagaacg

30

&lt;210&gt; 42

&lt;211&gt; 30

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Internal Primer RO1214

&lt;400&gt; 42

ggattcaatc atgtccaaag tccaaaccgc

30

&lt;210&gt; 43

&lt;211&gt; 34

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; 5' Primer RO1223

&lt;400&gt; 43

tctgatgaat tcatgacggc cggatttgaa gaag

34

&lt;210&gt; 44

&lt;211&gt; 35

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; 3' Primer RO1224

<400> 44  
 gtctagctcg agttagttct tgtcccaaggc aggca 35

<210> 45  
 <211> 1542  
 <212> RNA  
 <213> Thraustochytrium aureum

<400> 45  
 atgacggccg gatttgaaga agtgatcacc atgaaggcagg tgaaggaccg gaatacgccg 60  
 gacgatgcgt ggtgcgttgt gcatggcaag gtgtacgaca tcaccaagtt caagaacgct 120  
 caccgggtg gagatataat catgttggcg gctggcaagg acgccaccat cctgttcgag 180  
 acttaccaca tccgcgggtgt gcccgtatgcc gtgttgcgca agtacatcgat cggcaaactt 240  
 ccggacggaa agaacaaga gggcggcaac ggcctcgata ggcctcgta ctactcctgg 300  
 gacagcgagt tttaccgcgt ccttcgcgag cgcgtttga agcgcctgaa cgagctcaag 360  
 ctgtccagac gcggaggctt cgagatttgg gccaaggcta tctttctctt gaccggcttc 420  
 tggtcttgcc tctacacctat gtgcacactc aacccaaatg ggcttgcgat tcctgcgc 480  
 atgatgttgg gaatcttgc tgccctcgta ggaacctgca ttcagcacga cggaaatcac 540  
 ggtgcgttgc cccaatcttc gtggcttaac aaggccgctg gttggacttt ggacatgatt 600  
 ggatccagcg ccatgacctg ggagatgcag cacgtgttgc gacatcatcc gtacaccaac 660  
 ttgattgaaa tggagaatgg caatcaaaag gtctccggca agcctgttga caccaagact 720  
 gtcgaccagg agagcgaccc tgcgttgcgtt agcacctacc ctatgctcg ctttcaccct 780  
 tggcacagca aaaagtgtta ccacaaatac cagcacatct atgcaccatt catctttgg 840  
 ttcatgacca tcaacaaggt cattgcacag gacgttggcg ttatcacacg caagcgtctc 900  
 ttccagattt acgccaacttgc cgcctacgt tctccgactt acgtcgctcg cttctggatc 960  
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 ttcatcgtaa accacatcat cgagggtgtc agctacgcaa gcaaagatgc catcaaggc 1140  
 gagatggctc caccgaaaac ggtccgggt gtcaccccaa tgacagagac gcaaaagggtt 1200  
 ctcgaccaggc gcgagaaaaga catggacgaa acttctaaga agagccgcat ccctctcaac 1260  
 gactggccg ctgtacagtccagacc gtaacttggg ctatcggttc ttggttctgg 1320  
 aaccactttt ccgggggccc caatcatcg attgagacatc atctgttccc cggcttgcact 1380  
 cacaccacct atgttcaactt tcacgtatgt gtcacccatc ctgcgttgcgaa gtacgggtt 1440  
 ccataccaggc acgaggagag tctataacttgcctacttta agatgttgcgaa tcatctcaag 1500  
 accctaggca acgagccaat gcctgcctgg gacaagaact aa 1542

<210> 46  
 <211> 513  
 <212> PRT  
 <213> Thraustochytrium aureum

<400> 46  
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 Arg Asn Thr Pro Asp Asp Ala Trp Cys Val Val His Gly Lys Val Tyr  
 20 25 30  
 Asp Ile Thr Lys Phe Lys Asn Ala His Pro Gly Gly Asp Ile Ile Met  
 35 40 45  
 Leu Ala Ala Gly Lys Asp Ala Thr Ile Leu Phe Glu Thr Tyr His Ile  
 50 55 60  
 Arg Gly Val Pro Asp Ala Val Leu Arg Lys Tyr Gln Ile Gly Lys Leu  
 65 70 75 80  
 Pro Asp Gly Lys Asn Lys Glu Gly Gly Asn Gly Leu Asp Ser Ala Ser  
 85 90 95  
 Tyr Tyr Ser Trp Asp Ser Glu Phe Tyr Arg Val Leu Arg Glu Arg Val  
 100 105 110  
 Leu Lys Arg Leu Asn Glu Leu Lys Leu Ser Arg Arg Gly Gly Phe Glu

23/32

115	120	125	
Ile Trp Ala Lys Ala Ile Phe Leu Leu Thr Gly Phe Trp Ser Cys Leu			
130	135	140	
Tyr Leu Met Cys Thr Leu Asn Pro Asn Gly Leu Ala Ile Pro Ala Ala			
145	150	155	160
Met Met Leu Gly Ile Phe Ala Ala Phe Val Gly Thr Cys Ile Gln His			
165	170	175	
Asp Gly Asn His Gly Ala Phe Ala Gln Ser Ser Trp Leu Asn Lys Ala			
180	185	190	
Ala Gly Trp Thr Leu Asp Met Ile Gly Ser Ser Ala Met Thr Trp Glu			
195	200	205	
Met Gln His Val Leu Gly His His Pro Tyr Thr Asn Leu Ile Glu Met			
210	215	220	
Glu Asn Gly Asn Gln Lys Val Ser Gly Lys Pro Val Asp Thr Lys Thr			
225	230	235	240
Val Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Thr Tyr Pro Met Leu			
245	250	255	
Arg Leu His Pro Trp His Ser Lys Lys Trp Tyr His Lys Tyr Gln His			
260	265	270	
Ile Tyr Ala Pro Phe Ile Phe Gly Phe Met Thr Ile Asn Lys Val Ile			
275	280	285	
Ala Gln Asp Val Gly Val Ile Thr Arg Lys Arg Leu Phe Gln Ile Asp			
290	295	300	
Ala Asn Cys Arg Tyr Ala Ser Pro Thr Tyr Val Ala Arg Phe Trp Ile			
305	310	315	320
Met Lys Val Leu Thr Val Leu Tyr Met Val Gly Leu Pro Met Tyr Met			
325	330	335	
Gln Gly Pro Trp Glu Gly Leu Lys Leu Phe Phe Ile Ala His Phe Thr			
340	345	350	
Cys Gly Glu Leu Leu Ala Thr Met Phe Ile Val Asn His Ile Ile Glu			
355	360	365	
Gly Val Ser Tyr Ala Ser Lys Asp Ala Ile Lys Gly Glu Met Ala Pro			
370	375	380	
Pro Lys Thr Val Arg Gly Val Thr Pro Met His Glu Thr Gln Lys Val			
385	390	395	400
Leu Asp Gln Arg Glu Lys Asp Met Asp Glu Thr Ser Lys Lys Ser Arg			
405	410	415	
Ile Pro Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr Thr Val Asn			
420	425	430	
Trp Ala Ile Gly Ser Trp Phe Trp Asn His Phe Ser Gly Gly Leu Asn			
435	440	445	
His Gln Ile Glu His His Leu Phe Pro Gly Leu Thr His Thr Thr Tyr			
450	455	460	
Val His Phe His Asp Val Val Lys Asp Thr Cys Ala Glu Tyr Gly Val			
465	470	475	480
Pro Tyr Gln His Glu Glu Ser Leu Tyr Thr Ala Tyr Phe Lys Met Leu			
485	490	495	
Asn His Leu Lys Thr Leu Gly Asn Glu Pro Met Pro Ala Trp Asp Lys			
500	505	510	
Asn			

&lt;210&gt; 47

&lt;211&gt; 23

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

<220>  
<223> M13 Forward Primer

<400> 47  
agcggataac aatttcacac agg 23

<210> 48  
<211> 26  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer RO1270

<400> 48  
cacctggctc gagtcgacga tcatgg 26

<210> 49  
<211> 25  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer RO1286

<400> 49  
cgtacccggc gcaatagaag gtgag 25

<210> 50  
<211> 26  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer RO1287

<400> 50  
ccatcatcgt cgactcgagc caggta 26

<210> 51  
<211> 26  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer RO1288

<400> 51  
tgtggagcca tgtggtgctc gatctg 26

<210> 52  
<211> 42  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Forward Primer RO1400

<400> 52  
 tcaacagaat tcatgtgcaa cgccgcgcag gtcgagacgc ag 42  
  
 <210> 53  
 <211> 42  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Reverse Primer R01401  
  
 <400> 53  
 aaaagaaaagc ttttagtccg ctttgaccgt gtcgaccaaa gc 42  
  
 <210> 54  
 <211> 1301  
 <212> DNA  
 <213> Isochrysis galbana  
  
 <400> 54  
 atgtgcaacg cggcgcaggc cgagacgcag gccttgcgcg ccaaggaggc ggcaaaaccg 60  
 acgtggacga agattcatgg ggcacatgc gacgtggaga cgttccgcgc cccaggccgc 120  
 aacatccctcg atttgcgttccct gggcatggag ccacaactgc ctttgagacg ttccacggc 180  
 accacaaggg agcatgaaag atgctcaaga cgctgcccga gaaggaggc gcccggccgc 240  
 acatccccgc gcagaaggag gggcacgtgg cggagatgac acgcctcatg gcctcatggc 300  
 gcgagcgcgg gctgttcaag cccgcgtcccg tcgcctcatc catctatggc ctgtgcgtga 360  
 tcttcgccat cgcggcattcg gtcgcgtgcg ctccgtacgc gcaagtgcgt gctggcatcg 420  
 cggtggcgcac ctgctggcgt cagtgccgc tcttgcagca catggccgc caccggaggt 480  
 gggggcgcac ttggtcgttt gcgtttcagc atctgttga aggccgtc aaggccgcgt 540  
 cggcctcgtg gtggcgcaac cgcacacaaca agcaccatgc caagaccaac gtgctccgc 600  
 aggacggcga cctgcgcacc acacccttc tcgcattggg ccctactctg gccaagaaag 660  
 tgcccgactg gtctctgcgc acgcaagcct tcaccttct gccagcactg ggagcttacg 720  
 tcttcgtctt tgccttcacg gtacgcaagt acagtgttgtt gaagcgtc tggcacagg 780  
 tcgcctgtat ggtggccac tacgtctctt ttccctgggc gtcagcgcgc gcccggccct 840  
 ccctcagctc cggcctcacc ttctattgca cccggtaacgc ctgcaggccg atctaccccg 900  
 gcttcttctt cggcctatcg cacttgcgg tggagcgcgt gccgtcacc gccacctggc 960  
 tcgagtcgac gatgtatggc accgttgact gggccggcctc ctccgccttc tgcggctacc 1020  
 tctccggctt cctcaatatc cagatcgacg accacatggc tccacaaatg ccaatggaga 1080  
 acctgcgcac gatccggcc gactgcaagg cccggccca caagttcggg ctgcccgtacc 1140  
 gcgagctgac attcgtcgcg gcgaccaacg tcatgtatgag cggccctctac cggaccggca 1200  
 aggacgagct caagctgcgc gcgaccgc gcaagttcacc gaggccacag gctgtacatgg 1260  
 gcgccgcac cgctttggc gacacgctca aggccggacta a 1301  
  
 <210> 55  
 <211> 433  
 <212> PRT  
 <213> Isochrysis galbana  
  
 <400> 55  
 Met Cys Asn Ala Ala Gln Val Glu Thr Gln Ala Leu Arg Ala Lys Glu  
 1 5 10 15  
 Ala Ala Lys Pro Thr Trp Thr Lys Ile His Gly Arg Thr Val Asp Val  
 20 25 30  
 Glu Thr Phe Arg His Pro Gly Gly Asn Ile Leu Asp Leu Phe Leu Gly  
 35 40 45  
 Met Asp Ala Thr Thr Ala Phe Glu Thr Phe His Gly His His Lys Gly

50	55	60	
Ala Trp Lys Met Leu Lys	Thr Leu Pro Glu Lys	Glu Val Ala Ala Ala	
65	70	75	80
Asp Ile Pro Ala Gln Lys Glu Glu His	Val Ala Glu Met Thr Arg Leu		
85	90	95	
Met Ala Ser Trp Arg Glu Arg Gly	Leu Phe Lys Pro Arg Pro Val Ala		
100	105	110	
Ser Ser Ile Tyr Gly Leu Cys Val	Ile Phe Ala Ile Ala Ala Ser Val		
115	120	125	
Ala Cys Ala Pro Tyr Ala Pro Val	Leu Ala Gly Ile Ala Val Gly Thr		
130	135	140	
Cys Trp Ala Gln Cys Gly	Phe Leu Gln His Met Gly Gly His Arg Glu		
145	150	155	160
Trp Gly Arg Thr Trp Ser Phe Ala	Phe Gln His Leu Phe Glu Gly Leu		
165	170	175	
Leu Lys Gly Gly Ser Ala Ser Trp	Trp Arg Asn Arg His Asn Lys His		
180	185	190	
His Ala Lys Thr Asn Val	Leu Gly Glu Asp Gly Asp Leu Arg Thr Thr		
195	200	205	
Pro Phe Phe Ala Trp Asp Pro	Thr Leu Ala Lys Lys Val Pro Asp Trp		
210	215	220	
Ser Leu Arg Thr Gln Ala	Phe Thr Phe Leu Pro Ala Leu Gly Ala Tyr		
225	230	235	240
Val Phe Val Phe Ala Phe Thr Val	Arg Lys Tyr Ser Val Val Lys Arg		
245	250	255	
Leu Trp His Glu Val Ala	Leu Met Val Ala His Tyr Ala Leu Phe Ser		
260	265	270	
Trp Ala Leu Ser Ala Ala Gly	Ala Ser Leu Ser Ser Gly Leu Thr Phe		
275	280	285	
Tyr Cys Thr Gly Tyr Ala Trp	Gln Gly Ile Tyr Leu Gly Phe Phe Phe		
290	295	300	
Gly Leu Ser His Phe Ala Val	Glu Arg Val Pro Ser Thr Ala Thr Trp		
305	310	315	320
Leu Glu Ser Thr Met Met	Gly Thr Val Asp Trp Gly Gly Ser Ser Ala		
325	330	335	
Phe Cys Gly Tyr Leu Ser Gly	Phe Leu Asn Ile Gln Ile Glu His His		
340	345	350	
Met Ala Pro Gln Met Pro Met	Glu Asn Leu Arg Gln Ile Arg Ala Asp		
355	360	365	
Cys Lys Ala Ala Ala His	Lys Phe Gly Leu Pro Tyr Arg Glu Leu Thr		
370	375	380	
Phe Val Ala Ala Thr Lys	Leu Met Met Ser Gly Leu Tyr Arg Thr Gly		
385	390	395	400
Lys Asp Glu Leu Lys	Leu Arg Ala Asp Arg Arg Lys Phe Thr Arg Ala		
405	410	415	
Gln Ala Tyr Met Gly Ala Ala Ser Ala	Leu Val Asp Thr Leu Lys Ala		
420	425	430	
Asp			

<210> 56  
 <211> 14  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Protein Motif #1

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&lt;400&gt; 56

Val Tyr Asp Val Thr Glu Trp Val Lys Arg His Pro Gly Gly  
1 5 10

&lt;210&gt; 57

&lt;211&gt; 15

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Protein Motif #2

&lt;400&gt; 57

Gly Ala Ser Ala Asn Trp Trp Lys His Gln His Asn Val His His  
1 5 10 15

&lt;210&gt; 58

&lt;211&gt; 12

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Protein Motif #3

&lt;400&gt; 58

Asn Tyr Gln Ile Glu His His Leu Phe Pro Thr Met  
1 5 10

&lt;210&gt; 59

&lt;211&gt; 6

&lt;212&gt; PRT

&lt;213&gt; Thraustochytrium aureum

&lt;400&gt; 59

Gln His Asp Gly Ser His  
1 5

&lt;210&gt; 60

&lt;211&gt; 7

&lt;212&gt; PRT

&lt;213&gt; Thraustochytrium aureum

&lt;400&gt; 60

Gln His Val Leu Gly His His  
1 5

&lt;210&gt; 61

&lt;211&gt; 4

&lt;212&gt; PRT

&lt;213&gt; Thraustochytrium aureum

&lt;400&gt; 61

His Pro Trp His  
1

&lt;210&gt; 62

<211> 5  
<212> PRT  
<213> Thraustochytrium aureum

<400> 62  
His Lys Phe Gln His  
1 5

<210> 63  
<211> 6  
<212> PRT  
<213> Thraustochytrium aureum

<400> 63  
His Gln Ile Glu His His  
1 5

<210> 64  
<211> 7  
<212> PRT  
<213> Dictyostelium discoideum

<400> 64  
Gln His Val Ile Gly His His  
1 5

<210> 65  
<211> 6  
<212> PRT  
<213> Dictyostelium discoideum

<400> 65  
His Gln Val Val His His  
1 5

<210> 66  
<211> 7  
<212> PRT  
<213> Mortierella alpina

<400> 66  
Gln His Met Leu Gly His His  
1 5

<210> 67  
<211> 6  
<212> PRT  
<213> Unknown

<220>  
<223> Synechocytis (species unknown)

<400> 67  
His Gln Val Thr His His  
1 5

<210> 68

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<211> 335  
 <212> PRT  
 <213> Schizochytrium aggregatum

<220>  
 <221> VARIANT  
 <222> (335)...(335)  
 <223> Xaa = Unknown or other at position 335

<400> 68  
 Ser His Gly Ala Phe Ser Lys Ser Arg Phe Met Asn Lys Ala Ala Gly  
 1 5 10 15  
 Trp Thr Leu Asp Met Ile Gly Ala Ser Ala Met Thr Trp Glu Met Gln  
 20 25 30  
 His Val Leu Gly His His Pro Tyr Thr Asn Leu Ile Glu Met Glu Asn  
 35 40 45  
 Gly Leu Ala Lys Val Lys Gly Ala Asp Val Asp Pro Lys Lys Val Asp  
 50 55 60  
 Gln Glu Ser Asp Pro Asp Val Phe Ser Thr Tyr Pro Met Leu Arg Leu  
 65 70 75 80  
 His Pro Trp His Arg Gln Arg Phe Tyr His Lys Phe Gln His Leu Tyr  
 85 90 95  
 Ala Pro Leu Ile Phe Gly Phe Met Thr Ile Asn Lys Val Ile Ser Gln  
 100 105 110  
 Asp Val Gly Val Val Leu Arg Lys Arg Leu Phe Gln Ile Asp Ala Asn  
 115 120 125  
 Cys Arg Tyr Gly Ser Pro Trp Asn Val Ala Arg Phe Trp Ile Met Lys  
 130 135 140  
 Leu Leu Thr Thr Leu Tyr Met Val Ala Leu Pro Met Tyr Met Gln Gly  
 145 150 155 160  
 Pro Ala Gln Gly Leu Lys Leu Phe Phe Met Ala His Phe Thr Cys Gly  
 165 170 175  
 Glu Val Leu Ala Thr Met Phe Ile Val Asn His Ile Ile Glu Gly Val  
 180 185 190  
 Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val Met Ala Pro Pro Arg  
 195 200 205  
 Thr Val His Gly Val Thr Pro Met Gln Val Thr Gln Lys Ala Leu Ser  
 210 215 220  
 Ala Ala Glu Ser Thr Lys Ser Asp Ala Asp Lys Thr Thr Met Ile Pro  
 225 230 235 240  
 Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr Ser Val Asn Trp Ala  
 245 250 255  
 Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly Gly Leu Asn His Gln  
 260 265 270  
 Ile Glu His His Cys Phe Pro Gln Asn Pro His Thr Val Asn Val Tyr  
 275 280 285  
 Ile Ser Gly Ile Val Lys Glu Thr Cys Glu Glu Tyr Gly Val Pro Tyr  
 290 295 300  
 Gln Ala Glu Ile Ser Leu Phe Ser Ala Tyr Phe Lys Met Leu Ser His  
 305 310 315 320  
 Leu Arg Thr Leu Gly Asn Glu Asp Leu Thr Ala Trp Ser Thr Xaa  
 325 330 335

<210> 69  
 <211> 430  
 <212> PRT  
 <213> Isochrysis galbana

&lt;400&gt; 69

Met Cys Asn Ala Ala Gln Val Glu Thr Gln Ala Leu Arg Ala Lys Glu  
 1 5 10 15  
 Ala Ala Lys Pro Thr Trp Thr Lys Ile His Gly Arg Thr Val Asp Val  
 20 25 30  
 Glu Thr Phe Arg His Pro Gly Gly Asn Ile Leu Asp Leu Phe Leu Gly  
 35 40 45  
 Met Asp Ala Thr Thr Ala Phe Glu Thr Phe His Gly His His Lys Gly  
 50 55 60  
 Ala Trp Lys Met Leu Lys Thr Leu Pro Glu Lys Glu Val Ala Ala Ala  
 65 70 75 80  
 Asp Ile Pro Ala Gln Lys Glu Glu His Val Ala Glu Met Thr Arg Leu  
 85 90 95  
 Met Ala Ser Trp Arg Glu Arg Gly Leu Phe Lys Pro Arg Pro Val Ala  
 100 105 110  
 Ser Ser Ile Tyr Gly Leu Cys Val Ile Phe Ala Ile Ala Ala Ser Val  
 115 120 125  
 Ala Cys Ala Pro Tyr Ala Pro Val Leu Ala Gly Ile Ala Val Gly Thr  
 130 135 140  
 Cys Trp Ala Gln Cys Gly Phe Leu Gln His Met Gly Gly His Arg Glu  
 145 150 155 160  
 Trp Gly Arg Thr Trp Ser Phe Ala Phe Gln His Leu Phe Glu Gly Leu  
 165 170 175  
 Leu Lys Gly Gly Ser Ala Ser Trp Trp Arg Asn Arg His Asn Lys His  
 180 185 190  
 His Ala Lys Thr Asn Val Leu Gly Glu Asp Gly Asp Leu Arg Thr Thr  
 195 200 205  
 Pro Phe Phe Ala Trp Asp Pro Thr Leu Ala Lys Lys Val Pro Asp Trp  
 210 215 220  
 Ser Leu Arg Thr Gln Ala Phe Thr Phe Leu Pro Ala Leu Gly Ala Tyr  
 225 230 235 240  
 Val Phe Val Phe Ala Phe Thr Val Arg Lys Tyr Ser Val Val Lys Arg  
 245 250 255  
 Leu Trp His Glu Val Ala Leu Met Val Ala His Tyr Ala Leu Phe Ser  
 260 265 270  
 Trp Ala Leu Ser Ala Ala Gly Ala Ser Leu Ser Ser Gly Leu Thr Phe  
 275 280 285  
 Tyr Cys Thr Gly Tyr Ala Trp Gln Gly Ile Tyr Leu Gly Phe Phe Phe  
 290 295 300  
 Gly Leu Ser His Phe Ala Val Glu Arg Val Pro Ser Thr Ala Thr Trp  
 305 310 315 320  
 Leu Glu Ser Thr Met Met Gly Thr Val Asp Trp Gly Gly Ser Ser Ala  
 325 330 335  
 Phe Cys Gly Tyr Leu Ser Gly Phe Leu Asn Ile Gln Ile Glu His His  
 340 345 350  
 Met Ala Pro Gln Met Pro Met Glu Asn Leu Arg Gln Ile Arg Ala Asp  
 355 360 365  
 Cys Lys Ala Ala Ala His Lys Phe Gly Leu Pro Tyr Arg Glu Leu Thr  
 370 375 380  
 Phe Val Ala Ala Thr Lys Leu Met Met Ser Gly Leu Tyr Arg Thr Gly  
 385 390 395 400  
 Lys Asp Glu Leu Lys Leu Arg Ala Asp Arg Arg Lys Phe Thr Arg Ala  
 405 410 415  
 Gln Ala Tyr Met Gly Ala Ala Ser Ala Leu Val Asp Thr Leu  
 420 425 430

<210> 70  
<211> 515  
<212> PRT  
<213> *Thraustochytrium aureum*

<400> 70  
Met Thr Val Gly Phe Asp Glu Thr Val Thr Met Asp Thr Val Arg Asn  
1 5 10 15  
His Asn Met Pro Asp Asp Ala Trp Cys Ala Ile His Gly Thr Val Tyr  
20 25 30  
Asp Ile Thr Lys Phe Ser Lys Val His Pro Gly Gly Asp Ile Ile Met  
35 40 45  
Leu Ala Ala Gly Lys Glu Ala Thr Ile Leu Phe Glu Thr Tyr His Ile  
50 55 60  
Lys Gly Val Pro Asp Ala Val Leu Arg Lys Tyr Lys Val Gly Lys Leu  
65 70 75 80  
Pro Gln Gly Lys Lys Gly Glu Thr Ser His Met Pro Thr Gly Leu Asp  
85 90 95  
Ser Ala Ser Tyr Tyr Ser Trp Asp Ser Glu Phe Tyr Arg Val Leu Arg  
100 105 110  
Glu Arg Val Ala Lys Lys Leu Ala Glu Pro Gly Leu Met Gln Arg Ala  
115 120 125  
Arg Met Glu Leu Trp Ala Lys Ala Ile Phe Leu Leu Ala Gly Phe Trp  
130 135 140  
Gly Ser Leu Tyr Ala Met Cys Val Leu Asp Pro His Gly Gly Ala Met  
145 150 155 160  
Val Ala Ala Val Thr Leu Gly Val Phe Ala Ala Phe Val Gly Thr Cys  
165 170 175  
Ile Gln His Asp Gly Ser His Gly Ala Phe Ser Lys Ser Arg Phe Met  
180 185 190  
Asn Lys Ala Ala Gly Trp Thr Leu Asp Met Ile Gly Ala Ser Ala Met  
195 200 205  
Thr Trp Glu Met Gln His Val Leu Gly His His Pro Tyr Thr Asn Leu  
210 215 220  
Ile Glu Met Glu Asn Gly Leu Ala Lys Val Lys Gly Ala Asp Val Asp  
225 230 235 240  
Pro Lys Lys Val Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Thr Tyr  
245 250 255  
Pro Met Leu Arg Leu His Pro Trp His Arg Gln Arg Phe Tyr His Lys  
260 265 270  
Phe Gln His Leu Tyr Ala Pro Leu Ile Phe Gly Phe Met Thr Ile Asn  
275 280 285  
Lys Val Ile Ser Gln Asp Val Gly Val Val Leu Arg Lys Arg Leu Phe  
290 295 300  
Gln Ile Asp Ala Asn Cys Arg Tyr Gly Ser Pro Trp Asn Val Ala Arg  
305 310 315 320  
Phe Trp Ile Met Lys Leu Leu Thr Thr Leu Tyr Met Val Ala Leu Pro  
325 330 335  
Met Tyr Met Gln Gly Pro Ala Gln Gly Leu Lys Leu Phe Phe Met Ala  
340 345 350  
His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile Val Asn His  
355 360 365  
Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val  
370 375 380  
Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met Gln Val Thr  
385 390 395 400  
Gln Lys Ala Leu Ser Ala Ala Glu Ser Thr Lys Ser Asp Ala Asp Lys

	405	410	415												
Thr	Thr	Met	Ile	Pro	Leu	Asn	Asp	Trp	Ala	Ala	Val	Gln	Cys	Gln	Thr
	420				425								430		
Ser	Val	Asn	Trp	Ala	Val	Gly	Ser	Trp	Phe	Trp	Asn	His	Phe	Ser	Gly
	435					440						445			
Gly	Leu	Asn	His	Gln	Ile	Glu	His	His	Cys	Phe	Pro	Gln	Asn	Pro	His
	450				455				460						
Thr	Val	Asn	Val	Tyr	Ile	Ser	Gly	Ile	Val	Lys	Glu	Thr	Cys	Glu	Glu
	465				470				475			480			
Tyr	Gly	Val	Pro	Tyr	Gln	Ala	Glu	Ile	Ser	Leu	Phe	Ser	Ala	Tyr	Phe
					485				490			495			
Lys	Met	Leu	Ser	His	Leu	Arg	Thr	Leu	Gly	Asn	Glu	Asp	Leu	Thr	Ala
					500				505			510			
Trp	Ser	Thr													
	515														

<210> 71

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Histidine-Rich Motif

<400> 71

His Met Gly Gly His

1 5

<210> 72

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Histidine-Rich Motif

<400> 72

His Asn Lys His His

1 5

<210> 73

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Histidine-Rich Motif

<400> 73

Gln Ile Glu His His

1 5